The Fragmentation of Gondwanaland: Influence on the Historical Biogeography and Morphological Evolution within Dragon Lizards (Squamata: Agamidae)

By

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Abstract

Following the break up of the Pangaea, landmasses were aggregated into two super-continents, Laurasia in the northern hemisphere and Gondwanaland in the southern hemisphere. Both have been considered ancestral areas where many of today's taxonomic groups originated. However, during the Devonian, Gondwanaland began to fragment with micro-continental blocks breaking and rafting northwestwards across the Tethys Sea. These micro-continents eventually collided into Laurasia transforming into what today is Southeast Asia and parts of Wallacea. These micro-continental blocks carried Gondwanan lineages that evolved in isolation as they rafted across the Tethys Sea and subsequently dispersed into Laurasia. This geologic scenario has been used to explain why there are Asian lineages of lizards, birds, fish, and land snails that are more closely related to Australian and Papuan taxa than they are to other Asian lineages. One well-documented case of this biogeographic hypothesis is with Dragon Lizards. There have been two previous comprehensive studies on Dragon Lizard relationships, one based on discrete morphological characters and one using molecular data, and both predicted Gondwanan origins for all Dragon Lizards commensurate with the geologic scenario outlined above. Both studies, however, were based on limited sampling and reported problematic relationships that hindered their ability to unequivocally explain the geographic and evolutionary origins of this lizard family.

In this dissertation, I use phylogenomic data to resolve long-standing contentious Dragon Lizard relationships in order to revisit hypotheses of their Gondwanan origins and Tethys Sea migrations. I first use this phylogeny to test the hypothesis of an Indian (Gondwanan) origin for Southeast Asian Dragon Lizards and reevaluate the colonization of the Indian and the Indo-Himalaya regions. Once these problematic relationships and biogeographic origins were resolved, it enabled me to use unprecedented taxonomic sampling to provide the first estimate of a Dragon Lizard tree of life in order to test previous published hypotheses of Gondwanan origins and the morphological evolution within Dragon Lizards. Lastly, I use a dataset of 122 discrete morphological characters from a previous study and sum the number of unambiguous synapomorphies in the cranial and post-cranial skeletal characters associated with cephalic and axial body modifications in lineages that have morphological specializations in these regions. Lastly, I use the Dragon Lizard Tree of Life and these synapomorphies to perform a phylogenetic logistic regression analysis to show that the evolution of the higher number of relative synapomorphies associated with cephalic and axial body modifications are phylogenetically independent of one another. This study contributes to our knowledge of Gondwanan and Laurasian biogeography as well as how shared environmental pressures affect the external and internal morphology of unrelated species within Dragon lizards.

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Chapter 1

The Eurasian invasion: phylogenomic data reveal multiple Southeast Asian origins for Indian Dragon lizards

Jesse L. Grismer, James A. Schulte II, Alana Alexander, Philipp Wagner, Scott L. Travers, Matt D. Buehler, Luke J. Welton and Rafe M. Brown

Abstract

The Indian Tectonic Plate split from Gondwanaland approximately 120 MYA and set the Indian subcontinent on a ~ 100 million year collision course with Eurasia. Many phylogenetic studies have demonstrated the Indian subcontinent brought with it an array of endemic faunas that evolved *in situ* during its journey, suggesting this isolated subcontinent served as a source of biodiversity subsequent to its collision with Eurasia. However, recent molecular studies suggest that Eurasia may have served as the faunal source for some of India's biodiversity, colonizing the subcontinent through land bridges between India and Eurasia during the early to middle Eocene (~35–40 MYA). In this study we investigate whether the Draconinae subfamily of the lizard family Agamidae is of Eurasian or Indian origin, using a multi locus Sanger dataset and a novel dataset of 4,536 ultraconserved nuclear element loci. Results from our phylogenetic and biogeographic analyses revealed support for two independent colonizations of India from Eurasian ancestors during the early to late Eocene prior to the subcontinent's hard collision with Eurasia. These results are consistent with other faunal groups and new geologic models that suggest ephemeral Eocene land bridges may have allowed for

dispersal and exchange of floras and faunas between India and Eurasia during the Eocene.

Introduction

The collision of the Indian subcontinent (ISC) into Eurasia caused the formation of some of the world's most iconic deserts and mountain ranges, dramatically changing Asian climates, while simultaneously sculpting its biodiversity. Much interest has centered on investigating the evolutionary and geological processes that have influenced the origins and diversification of the ISC's unique biotas ([Karanth 2006; and references therein). Phylogenetic studies of birds, dipterocarp trees, terrestrial gastropods, crabs, freshwater fish, and certain groups of amphibians, suggests these lineages originated on the ISC and were a source of biodiversity for regions of Asia and areas as far west as Africa after the Indian Plate split off from Gondwanaland [Dayanandan et al. 1999; Bossuyt and Milinkovitch 2001; Gower eta l. 2002; Sparks 2003; Dutta et al. 2004; Klaus et al. 2010). However, a suite of phylogenetic studies across a variety of other taxa suggest an alternative biogeographic hypothesis postulating Eurasia as the ancestral source of diversity for the ISC. In these groups Asian lineages dispersed to, and successfully colonized, the subcontinent before its hard collision with Eurasia 25–30 MYA [Raxworthy et al. 2002; Renner 2004; Köhler and Glaubrecht 2007; van der Meijden; Macey et al. 2008].

The previous lack of geologic models describing the fine scale events of the final 50 million years of the ISC's collision, left researchers with no mechanistic explanation for the striking differences between these two "ISC faunal origin" hypotheses. Fortunately, newer models are available that take into account continental connections between the approaching ISC and areas of mainland Asia prior to the ISC's collision with Eurasia [Acton 1999; Aitchison et al. (2007); Ali and Aitchison 2008]. Acton (1999) and Ali and Aitchison (2008) hypothesized that between 34–55 MYA (middle Eocene-late Eocene), India was connected to Eurasia via land-bridges with Sumatra, and then along what is now the Thai-Malay Peninsula and Burma (which would have been one land mass during this time). Two recent studies have recovered phylogenetic support for these Eocene land bridges and hypothesized that these pre-collision continental connections would have allowed for faunal exchanges between the ISC and Eurasia as the ISC continued northward [Klaus et al. 2010; Li et al. 2013]. We present data from a diverse radiation of Indian and Southeast Asian lizards that provide an additional model system, with larger amounts of generic diversity of Indian lineages and Asian lineages, to test for phylogenetic support for these Eocene land bridges, which we refer to as the Eocene Exchange Hypothesis (EEH).

The Draconinae is a subfamily within the lizard family Agamidae that contains 27 genera and 199 species [Manthey 2008] comprising approximately 50% of total Agamid diversity. Members of the Draconinae collectively range throughout mainland Asia (Indochina), Sundaland, India, and Sri Lanka (Fig. 1.1). Draconinae lizards are diurnal omnivores exhibiting a range of arboreal and terrestrial life styles and are some of the dominant members of diurnal lizard communities throughout South and Southeast Asia [Manthey and Grossmann 1997; Malkmus et al. 2002]. To date, only two studies have investigated the phylogenetic relationships within the Draconinae. However, both were part of broader systematic studies on the entire Agamidae family [Moody 1980; Macey et al. 2000]. Moody's (1980) dissertation included 60 extant taxa, was based on

122 morphological characters, and included data from 18 fossils. This work was the first study to hypothesize a Eurasian origin for the Indian draconine lineages. Macey et al. (2000) was the first study to provide a molecular phylogeny for the Agamidae (including Draconinae), and included an analysis of 72 taxa and one mitochondrial gene. This analysis demonstrated that mainland Asian agamids were paraphyletic with respect to Indian and Sri Lankan lineages. However, multiple deeper nodes within the Draconinae were characterized by poor support, resulting in ambiguous relationships [Macey et al. 2000]. The authors then used a series of parsimony methods to suggest that these problematic areas of the draconine phylogeny, along with a lack of biogeographic signal, were likely due to an Indian-Asian faunal exchange just after the hard collision, 20–25 MYA. Subsequent reviews of Indian-Eurasian collision regarded the biogeographical interpretations of Macey et al. [Macey et al. 2000] with skepticism due to the poorly supported relationships within the Draconinae [Datta-Roy and Karanth (2009); and references therein].

Since Moody(1980) and Macey et al. (2000), new Draconinae genera have been discovered, and previously unsampled rare genera have been collected, providing additional genetic material for reanalysis of draconine relationships. The lower per-base cost of next-generation sequencing has also led to the development of genomic methods extending the number of genetic markers that have limited the phylogenetic resolution in previous studies. Here, we generate a genomic data set of 4,536 nuclear loci derived from ultraconserved elements (UCEs), along with traditional Sanger sequencing data, to resolve the problematic relationships within the Draconinae reported by Macey et al. (2002). With the addition of new taxa, and genomic sequence-capture data, analyzed in combination with newly developed geological models, we are poised to reinterpret the biogeographic origins of Indian and Southeast Asian draconine lineages. Specifically, we tested **(1)** Moody's (1980) pre-collision hypothesis versus Macey et al. (2002) post-collision hypothesis for the origins of Indian lineages; and **(2)** suggest that a conclusion in favor of Moody's (1980) pre-collision hypothesis would show phylogenetic support for the Eocene land bridge connections proposed by Acton (1999) and Atchison et al. (2007). We term this the Eocene Exchange Hypothesis (EEH).

Methods

DNA Extraction, Sanger Mitochondrial and Nuclear DNA Sequence Data Collection

Genomic DNA was extracted from muscle or liver tissue samples on loan form La Sierra University, Villanova University, the California Academy of Sciences, the Zoologisches Forschungsmuseum Alexander Koenig, and the Chicago Field Museum. Extractions were preformed using a DNeasy tissue kit (Qiagen, Inc.) and sequenced for the mitochondrial and nuclear genes, ND2 (primers from Macey et al (2000) and RAG-1 (primers from [Melvile et al. 2009]), respectively, using standard PCR and Sanger sequencing protocols. We edited the sequences and aligned them within Geneious Pro 5.0.4 (http://www.geneious.com, [Kearse et al. 2012]) and these new sequence data were combined with existing data from [Macey et al. 2000] and [Melvile et al. 2009] (Appendix 1). In total, the dataset included 17 of the 26 draconine genera, including all but two of the Indian genera (*Psammophilus* and *Coryphophylax*). *Hyrdosaurus* and *Physignathus* were not included as their phylogenetic affinities are with other agamid lineages outside of the Draconinae [Macey et al. 2000]. At least three species (or individuals if the genus was monotypic) per genus were sampled, for a total of 44 individuals. ND2 and RAG-1 were selected as they are the most frequently sequenced markers across acrodont lizards and therefore provide maximum taxonomic coverage. We used these markers to preliminarily place new genera in a phylogenetic context, and as a guide tree in our selection of genera for UCE development to resolve problematic relationships.

Ultraconserved elements (UCE) data collection

To resolve the problematic areas in the phylogeny from the Sanger data (pink nodes: Fig 1.2A), we selected 24 individuals representing 12 genera (underlined taxon names in Fig. 1.2) from across four species groups (brown nodes: Fig. 1.2A) for ultaconserved element (UCE) enrichment. Sequence-capture data collection followed a modification of the approach outlined by Faircloth et al. (2012). Briefly, we fragmented genomic DNA with a Covaris S220 ultrasonicator (Covaris, Inc.), and prepared Illumina libraries using KAPA library preparation kits (Kapa Biosystems) and custom sequence tags unique to each sample [Faircloth and Glenn 2012]. Libraries were pooled into groups of 8 taxa and enriched for 5,060 UCE loci (5,472 probes). We amplified enriched pools with a limited-cycle PCR (18 cycles) and sequenced final libraries on a partial Ilumina HiSeq 2000 lane. Reads were quality filtered using the Illumiprocessor [Faircloth et al. 2013] wrapper for Trimmomatric [Bolger et al. 2013], and assembled into contigs using Trinity [Grabherr et al. 2011]. Where alternate alleles differing by less than 5% sequence divergence (or two nucleotide positions, whatever was greater) were present in a sample for any given UCE locus, Trinity retained the allele supported by the largest number of reads. We used PHYLUCE v. 1.4 (Faircloth et al. 2012; (Faircloth et al. 2014] to match contigs to UCE loci and generated two alignments in MAFFT [Katoh et al. 2009]: one containing no missing loci across all individuals (complete) and another containing data for at least 75% of taxa per locus (75% complete), which returned alignments of 1114 loci and 4,536 loci, respectively.

Phylogenetic and Biogeographic Analyses

We first used Bayesian analyses with MrBayes 3.2.2 [Ronquist et al. 2012] of the ND2 and RAG-1 datasets independently in the context of the entire Agamidae to ensure that Draconinae was monophyletic. Once monophyly and lack of conflict between loci was established, we concatenated the two gene partitions for subsequent analyses. We used uniform priors in MrBayes 3.2.2 and partitioned the dataset by locus and codon within each locus for just the members Draconinae sub-family. We then assigned the GTR+ Γ substitution model for each partition and used three chains (two hot and one cold), and carried out 100 million generations, sampled every 10,000 generations. Due to the risk of substitution saturation, we performed analyses including and excluding the third codon position for the ND2 alignment. Convergence between chains, likelihood scores, and estimate sample size (ESS) values were evaluated using Tracer 1.6 [Rambaut et al. 2014] In order to obtain a reliable root age for divergence-time estimates within Draconinae, we expanded our ND2 and RAG-1 datasets to include data from all acrodont lineages. We analyzed this expanded dataset using eight acrodont fossils (Appendix 2) within a Bayesian framework in BEAST 2.3 [Bouckaert et al. 2014] using the fossilized-birth-death model [Stadler 2010; Heath et al. 2014]. The fossilized-birth-death process provides a model for the distribution of speciation times, tree topology, and distribution of lineages sampled before the present, and treats the fossil observations as part of the prior on node time estimates. We used the root age for the Draconinae resulting from this analysis (85 MYA) as a minimum-age calibration for the root of the Draconinae for subsequent time of divergence estimates within the Draconinae clade.

We then performed likelihood analyses in RAxML v.8.1.20 [Stamatakis 2014] on concatenated datasets for the incomplete (4,536 loci) and complete (1,114 loci) matrices, using the GTR+ Γ substitution model, and ran 100 fast bootstrap replicates. In addition to the concatenated analysis, maximum likelihood gene trees were constructed for each of the UCE loci included in the complete matrix using Phyluce with RAxML v.8.1.20 [Stamatakis 2014], under default settings. Phyluce and RAxML were also used to generate gene trees for 500 multi-locus bootstraps [Seo 2008]. Custom R-scripts (R v3.2.0; R Core Team 2015) and the R library Phybase [Liu and Yu 2010] were then used to infer the STEAC [Li et al. 2009] summary species tree for the original and bootstrapped data.

Using 85 MYA as a minimum age limit for the ancestor of the Draconinae, divergence dates for subclades were estimated in BEAST 2.3 using the ND2 and RAG-1 datasets with linked clock and tree models. We applied Birth-Death tree priors and constrained the relationships to match the results from the analyses of the UCE loci (blue nodes: Fig. 1.2B) and let the relationships within each species group be estimated by the BEAST analyses. We used a relaxed uncorrelated lognormal clock model and an exponential prior for the mean rate of each partition. Default values were used for all other priors, and the analysis was run for 150 million generations sampling every 12,000 generations, with chain stationarity, and ESS values were evaluated in Tracer 1.6. The first 25% of trees were discarded as burn-in and the maximum clade credibility tree with median node heights was summarized using TreeAnnotator 2.3 Bouckaert et al. 2014]. We converted our alignments to fasta format using seqmagick (http://seqmagick.readthedocs.org/en/latest/). Then, with the estimate for divergence between *Mantheyus* and other draconine species of 85MYA, we estimated the TMRCA of subclades based on pairwise Hamming distances [Hamming 1950] between UCE loci (with a sequence saturation correction of 0.95) calculated through fastphylo [Khan et al. 2013], assuming a naïve strict clock. We carried out the calculations using a custom R-script [Alexander 2015]. Any loci where subgroup divergence times exceeded those of the calibration time were discarded due to the likelihood of incomplete lineage sorting and/or excessive rate variation. Using the same methods, we then estimated the time to most recent common ancestor (TMRCA) of the *Draco*+*Ptyctolaemus* and species group 1-4 clades using the estimated age of the Non-*Mantheyus* clade. The estimate of the TMRCA of species group 1-4 was then used to age the split between *Acanthosaura* and *Pseudocalates* (species group 1), and the ancestor of species groups 2/3/4. The species group 2/3/4 TMRCA estimate was then used to age the split between *Salea* and *Calotes* (species group 2 and 3), and the ancestor of species group 4. Finally, the estimate for the TMRCA of species group 4 was used to obtain an estimate of the TMRCA of *Certaophora/Lyriocephalus/Cophotis*.

Ancestral area reconstructions were performed using likelihood and Bayesian methods in LAGRANGE within the program RASP 3.0 [Yu et al. 2014], and in RevBayes 10.10 [Hohna et al. 2014] respectively. Taxa were assigned to their biogeographic zone (Fig 1) based on their modern day distributions and RevBayes reconstructions were visualized using the online resource Phylowood [Landis and

Bedford 2014]. Traditionally, the Philippines is not classified as part of Sundaland however, we included taxa from this archipelago in the Sundaland biogeographic area because the entire Philippine agamid fauna is Sundaic in origin.

Results

Sanger Mitochondrial and Nuclear Data Phylogenetic Analyses

The Bayesian analyses of the combined Sanger dataset recovered new relationships that have not been reported in any previous study (Fig. 1.2A). *Mantheyus* was recovered as sister to the remaining Draconinae. The next lineage to diverge was a well-supported clade containing *Draco,* and *Ptyctolaemus* (Fig. 1.2A). Lastly, there were four well-supported species groups (brown nodes: Fig. 1.2A). The relationships within each of these species groups were well supported. However, the relationships between the species groups were poorly resolved and characterized by short branches (pink nodes: Fig. 1.2A). As the resolution of the relationships between the species groups is vital for testing hypotheses of Indian or Eurasian origins, representatives of the taxa from each of these species groups were included in a phylogenetic reconstruction from analyses of UCE data.

Sequence-capture data phylogenetic analyses

There were 4,536 loci with data for at least 75% of the n=23 individuals included in this study. These loci had an average length of 644.7 bp (S.D. = 249.7 bp), of which an average of 10.5% of sites (S.D. = 20.0%) were parsimony informative. The average amount of missing data per locus was 23.6% (S.D. = 19.4%), including both missing individuals (up to 25% of individuals at each locus) and shorter sequence lengths for individuals that were present (Table. S3). All analyses of the sequence-capture data were successful in resolving the problematic relationships recovered from the Sanger data (blue nodes; Fig. 1.2B) and recovered each of the four species groups within the Draconinae, with high support (brown nodes; Fig.2B), consistent with the results from the Sanger datasets.

Biogeographic analyses, divergence dating, and ancestral areas

Both of the methods employed to estimate ancestral ranges (LAGRANGE and RevBayes analyses) returned comparable estimates of ancestral areas, however, the RevBayes reconstructions were more conservative. Given the short branch lengths leading to some of the deeper nodes in our phylogeny, the RevBayes reconstructions are a better reflection of geology at the times of these nodes. Therefore only the RevBayes reconstructions are discussed. The grafted BEAST time-tree (Fig. 1.3A) was concordant with the phylogenies derived from the Sequence capture data and Sanger data (Fig. 1.2). The BEAST time-tree (Fig. 1.3) indicated the most recent common ancestor (MRCA) for the Draconinae originated approximately 92 MYA in mainland Asia ~30 million years after the ISC broke off Gondwanaland. The MRCA for *Draco* and its relatives most likely originated in mainland Asia 53 MYA and diverged from the other mainland Asian and Sundaic lineages around 69 MYA from a mainland Asian ancestor. The three remaining species groups appear to have diversified from one another rapidly between 51–59 MYA, most likely from a mainland Asia ancestor that existed approximately 59 MYA. The Indian endemic *Salea* (Species group 2) represents the first invasion of India (D#1: Fig. 1.3A), having diverged from a mainland Asian ancestor it shared with *Calotes* (Species group 3) approximately 56 MYA (Fig. 1.3A). The MRCA for *Acanthosaura* and *Psuedocalotes* (species group 1) was estimated at 56 MYA with a high probability that this ancestor originated in either mainland Asia or Sundaland (where both genera presently occur). Within this species group, we recovered support for a second invasion of India and Sri Lanka, with the ancestor of *Sitana* and *Otocryptis* originating from a predominantly Sundaic ancestor between 51–27 MYA (D#2: Fig. 1.3A). Lastly, the MRCA for the Sri Lankan and Sundaland radiations (species group 4) originated around 51 MYA in Sundaland or Sri Lanka (Fig. 1.3A). Within species Group 4, *Aphaniotis, Bronchocela,* and *Gonocephalus* appear to have diverged from one another 42 MYA and form the sister lineage to the Sri Lankan genera *Lyriocephalus, Cophotis,* and *Ceratophora* (Fig. 1.3A). The Sri Lankan lineages diverged from one another 28 MYA. We obtained these timing estimates for key divergences and dispersal events using Sanger data (as they were available for a broader taxonomic sample, including key fossils in comparison with the UCE data) in BEAST, with the topology constrained by the results from UCE data. We then crosschecked these estimates using the minimum divergence time for Draconinae of 85 MYA, and sequence divergence among UCE loci between clades of interest. This method is somewhat cruder than the BEAST estimates because it cannot account for among lineage rate variation. However, the estimates obtained using this approach were broadly comparable with results or our Bayesian analysis performed in BEAST (Fig. 1.4), offering support for our timing of key draconine dispersal events in Southeast Asia.

Discussion

In this study, we utilized unprecedented sampling of the Draconinae, both in taxonomic diversity and genetic markers, to give fresh biogeographic insight into the origins of the Indian and Southeast Asian Draconinae lineages. In particular, the thousands of loci generated using sequence-capture and next-generation sequencing were successful in resolving previously problematic relationships within the Draconinae (brown nodes: Fig. 1.2). Using the fully resolved UCE phylogeny to constrain the topology of our Sanger dataset, we generated a grafted Bayesian time tree (Fig. 1.3A), which supported the hypothesis that there were at least two independent colonization events of India by Southeast Asian lineages during the Eocene. These results favor Moody's (1980) pre-collision hypothesis with the estimated times of the Eurasian invasions in accordance with the Eocene land bridges proposed by Acton, (2007) and Ali and Aitchison (2008). These hypothesized land bridges would have connected areas of Eurasia (now Sundaland and the Thai-Malay peninsula) and the ISC before its collision, and are the likely conduits for terrestrial faunal exchange and range expansion in the lineages leading to today's Indian subcontinent endemics *Salea, Sitana,* and *Otocryptis*.

The Eocene Exchange Hypothesis

The first Draconinae invasion into India consisted of a lineage represented today by the endemic genus *Salea,* which descended from a mainland Asian ancestor that also gave rise to the Indochinese genus *Calotes*. This colonization event most likely resulted from an early Eocene land-bridge connection or an over-water dispersal event just prior to the ISC's connection with Sundaland (Eurasia) 50–55 MYA (Fig. 1.3B). Given the sedentary and arboreal natural histories of extant draconine species, we feel the former hypothesis is more likely than the latter, although we acknowledge the possibility of both. We expect a broader sampling within this clade of Southeast Asian, and especially Indian, species will provide a better estimate of the ancestral area at this node (*Salea* +

Calotes: Fig. 1.3A). The second dispersal event into India occurred with the divergence of the Indian and Sri Lankan endemics *Sitana* and *Otocryptis* from an ancestor most likely found in Sundaland during the middle Eocene. This colonization of the Indian subcontinent most likely was facilitated via a land bridge that connected the ISC with Sumatra and the Thai-Malay peninsula at 48 MYA. Additionally, the lineage sister to *Sitana* and *Otocryptis, Japalura,* and *Pseudocalotes,* is *Phoxophrys* (Fig. 1.3A). This genus is endemic to the lowland forests of Borneo and Sumatra—further supporting an India-Sundaland (Eurasia) connection via Sumatra and the southern portion of the Thai-Malay Peninsula during the middle Eocene. These independent colonization events not only support Moody's (1980) pre-collision biogeographic hypothesis, but also give additional phylogenetic support for Eocene land bridges postulated by Acton, (1999) and Ali and Aitchison (2008). Our results contribute to a growing body of literature demonstrating the possibility of floral and faunal exchange between India and Eurasia during the Eocene, before the ISC's hard collision 20–25 MYA (e.g. freshwater crabs: [Klaus et al. 2010]; rhacophorid tree frogs: Li et al. [2013]). Given the ecology of these organisms, and of the draconine species sampled here, we feel that it is less likely Eocene faunal exchanges occurred as the result of over water dispersal events. It is unclear whether the Eocene land bridges were two separate spatial/temporal features, versus possibly the same entity, just changing position as the ISC progressed northward. In either case, their existence may have provided continental connections between Southeast Asia and India during the Eocene, which could have allowed for terrestrial exchanges between these areas. These results collectively represent a broad-scale pattern of faunal exchange between the ISC and areas of Eurasia before its collision with Asia, at least partially facilitated by land bridges, which we term the "Eocene Exchange Hypothesis." Furthermore, we believe the reoccurring and somewhat subjective disagreement between the Indian vs. Asian origins hypotheses [Dayanandan et al. 1999; Bossuyt and Milinkovitch 2001; Gower eta l. 2002; Sparks 2003; Dutta et al. 2004; Klaus et al. 2010; Raxworthy et al. 2002; Renner 2004; Köhler and Glaubrecht 2007; van der Meijden; Macey et al. 1998; Li et al. 2013], have simply identified opposing perspectives of a broad geographic and temporal conduit of opportunity for faunal exchange between India and Eurasia. Future studies would benefit from an attempt to empirically focus on the timing and direction of faunal exchange between these biogeographic regions, rather than a prevalence of one scenario over the other.

Revision of the age of Draconinae

Our estimate for the age of Draconinae is significantly older than those previously published in broad scale squamate phylogenetic studies (most recently [Reeder et al. 2015]). Our older estimates are largely due to our consideration of the acrodont fossils, *Mimeosaurus* and *Priscagama*, as leiolepids rather than stem agamids, following Estes et al. 1988. These fossils have had a rather turbulent history of classification, with various studies suggesting *Mimeosaurus* was allied with the Chameleonidae [Gilmore 1943]; then hypothesized to be located along the branch leading to *Leiolepis* and *Uromystax* [Moody 1980]; and lastly united with *Priscagama* in an extinct subfamily, Priscagaminae [Borsuk-Bialynicka and Moody 1984], considered to be a stem lineage of *Leiolepis* and *Uromystax* [Gao and Norell 2000].

This confusion has persisted because when *Mimeosaurus* and *Priscagama* were first described, the contemporary genera *Leiolepis* and *Uromystax* were still included within the family Agamidae and demonstrated to be the sister group to the remaining agamids [Moody 1980] (this relationship has been further confirmed with molecular data [Macey et al. 2000; Melvile et al. 2009; Townsend et al. 2004; Grismer and Grismer 2010]. However, Estes et al. (1988) removed *Leiolepis* and *Uromystax* from the Agamidae and placed them in their own family (the Leiolepidae), and this taxonomy has not been followed by subsequent studies. Thus, the acrodont fossils of *Priscagama* and *Mimeosaurus* have been consistently considered as stem fossils for all agamids and not their sister group, *Leiolepis* and *Uromystax*. We followed the taxonomy of Estes et al. (1988) and considered *Mimeosaurus* and *Priscagama* as stem leiolepids and not stem agamids. It was this placement that lead to our older estimates of Draconinae origins (85–92 MYA). However, this estimate is consistent with the ages of new amber agamid fossils being described out of Indochina and previous studies on Iguanian lizards ([Schulte and Moreno-Roark 2010]; Bauer et al., unpublished data; *personal communication with JLG and PW*). We recommend that researchers continue to follow the taxonomy of [Estes et al. 1988] with the recognition of the Leiolepidae as a distinct family and the placement of priscagamine fossils as stem to *Leiolepis* and *Uromystax*, as suggested in the original descriptions of these fossils [Moody 1980; Borsuk-Bialynicka and Moody 1984; Gao and Norell 2000].

Conclusions

The use of additional taxa, sequence-capture data, and newer geological models—all data not available to previous studies on Draconinae—resulted in novel and well-resolved relationships, leading to new biogeographic insights in this unique subfamily of lizards. Using these biogeographic insights and a broad comparison with previous biogeographic literature, we propose the Eocene Exchange Hypothesis, and the simple but well supported assumption that land bridges may have facilitated a broad-scale pattern of faunal exchange between the ISC and areas of Eurasia before its collision with Asia during the Eocene. We expect that with additional sampling of Indian and mainland Asian species, some factors that may have biased our biogeographic interpretations within the Draconinae to (i.e., Indian extinction events), can be evaluated. In addition, sampling of additional draconine species will allow us to test more fine-scaled hypotheses concerning dispersal and diversification within this group. Our phylogenomic analysis add to a growing body of knowledge addressing the effects of the ISC's collision on biogeography and offers new ideas to be tested by future studies.

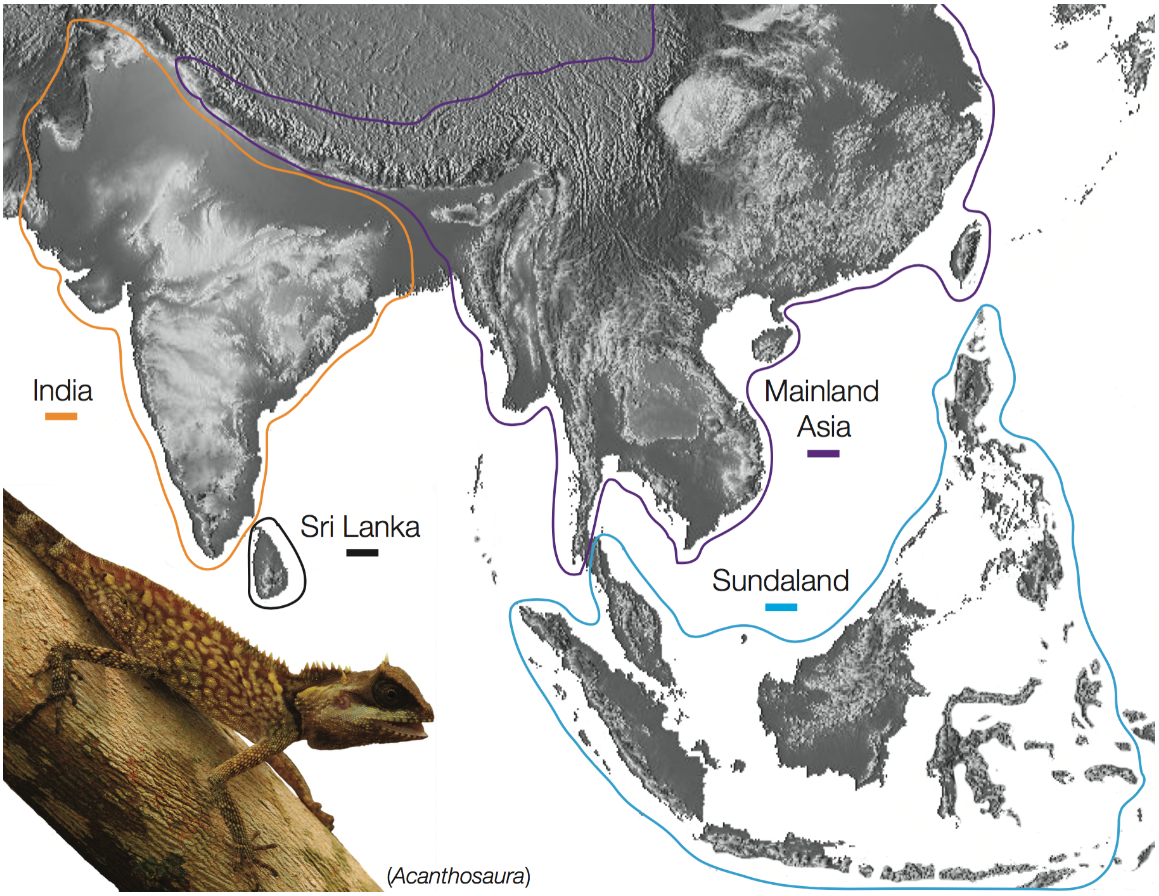

Figure 1.1– Map showing the distribution of Draconinae and the four biogeographic area (differently-colored borders) used in ancestral range reconstructions.

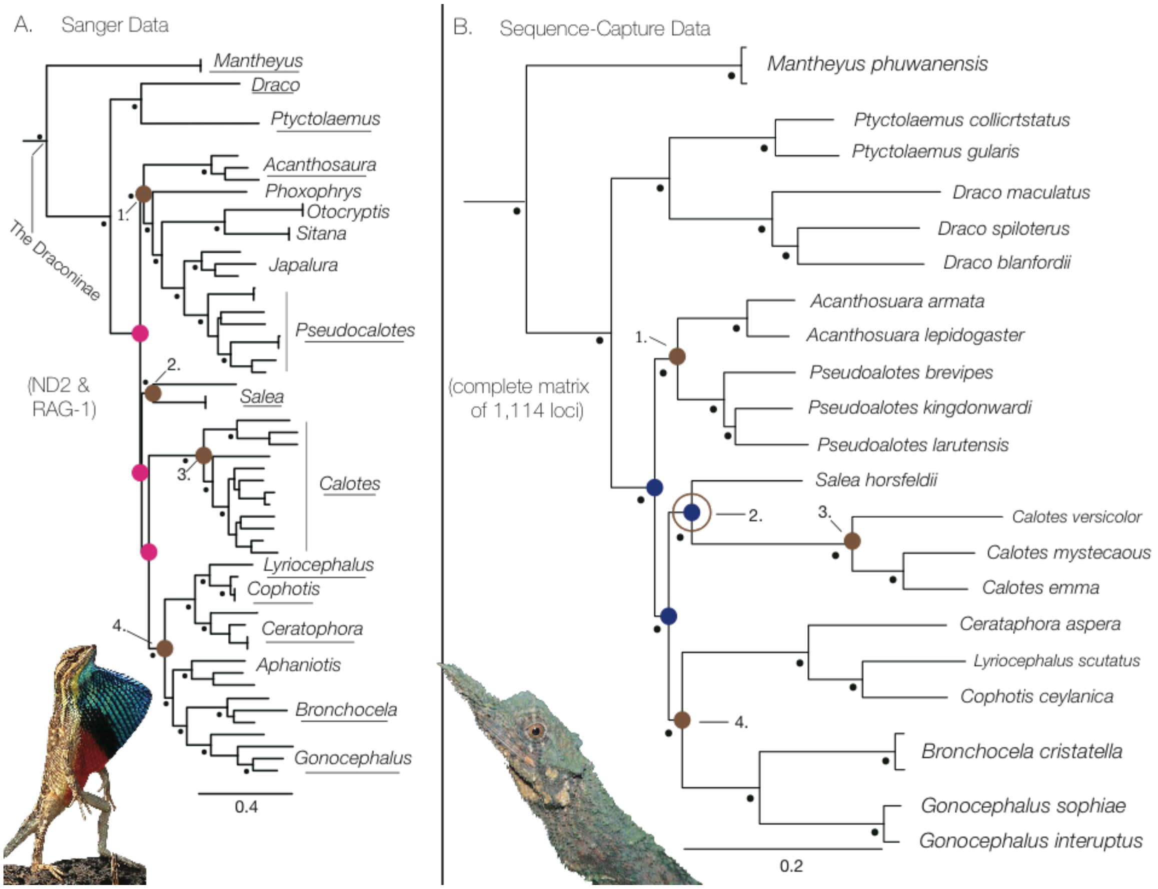

Figure 1.2 – (A). Bayesian analysis (in MrBayes) of ND2 and RAG-1 data, with black dots denoting nodes with posterior probabilities above 0.95. Brown nodes indicate four well-supported species groups (1–4; see text for details) and pink nodes identify poorly supported relationships among these species groups. Underlined taxon names are genera selected for UCE enrichment. (B). Multi-species coalescent ("species tree") from the species tree estimation using average coalescence times STEAC analysis, using the complete matrix of 1,114 UCE loci. Black dots denote nodes with 100 bootstrap support. Brown nodes indicate the four species groups (Group 2=brown circle; see text for discussion). Blue nodes identify problematic nodes recovered in Likelihood analysis of the Sanger dataset, resolved with sequence-capture data.

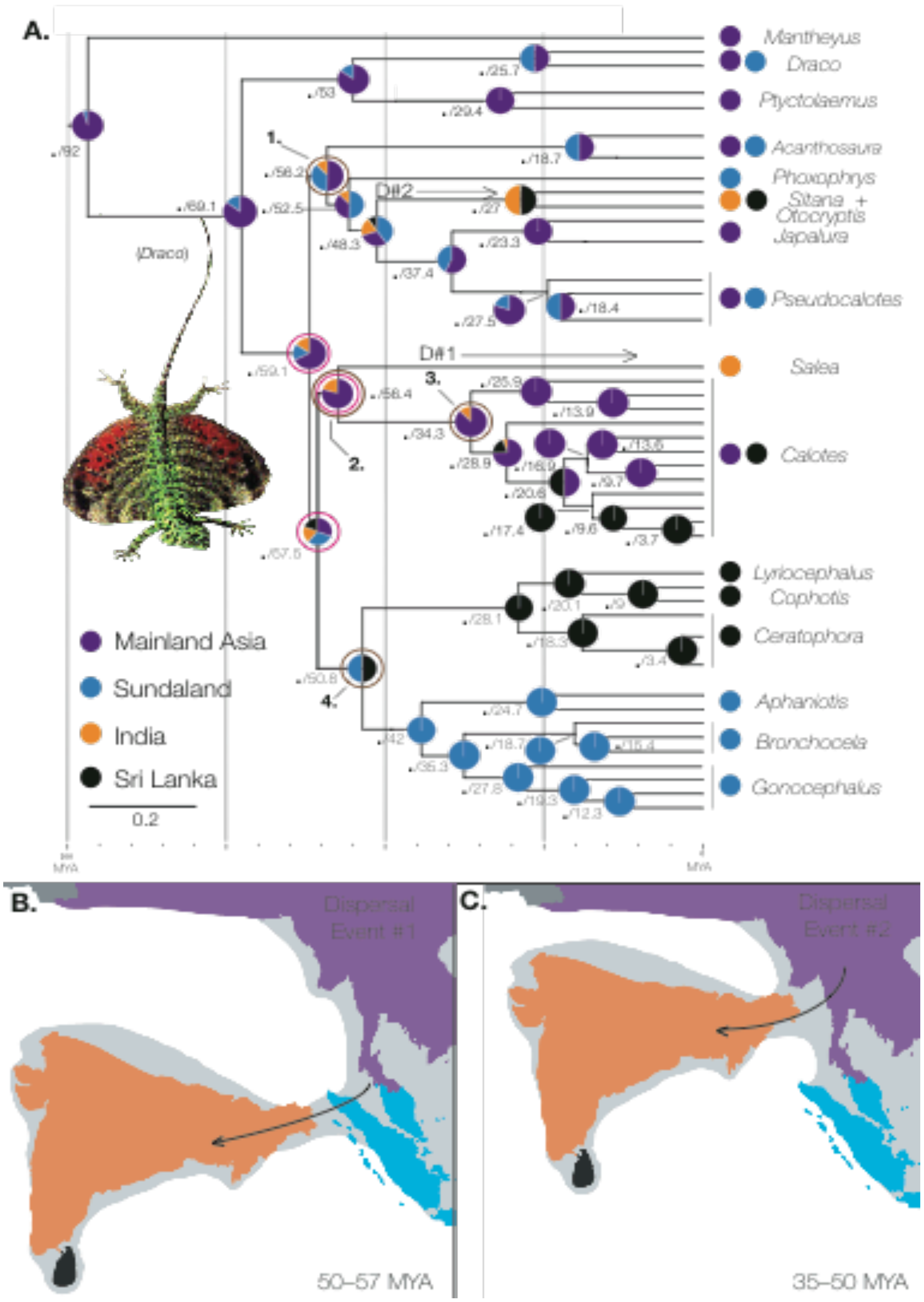

Figure 1.3 – (A). Time-calibrated Bayesian analysis of ND2 and RAG-1 data, with black dots denoting nodes with posterior probabilities above 0.95, followed by the estimated divergence time for each node in MYA. Pink circles identify nodes where topology was constrained based on Likelihood and species tree analyses of UCE data (Fig. 1.2B).

Brown circles indicate the four species groups. Biogeographic distributions of contemporary samples follow area coding depicted in Figure 1, with probability of areas at ancestral nodes from our Bayesian analysis in RevBayes. Inferred dispersal events into India are labeled D#1 and D#2, resulting in Indian or Indian/Sri Lankan *Salea, Sitana,* and *Otocryptis*. (B). Hypothesized position of the ISC and an early Eocene land bridge allowing for the first inferred dispersal event (D#1 in Fig. 1.3A) from Eurasia into India, 50–55 MYA. (C). Hypothesized position of the ISC and a middle-late Eocene land bridge allowing for the second first inferred dispersal event (D#2 in Fig. 1.3A) from Eurasia into India between 35–50 MYA (paleomaps modified from Klaus et al. 2010).

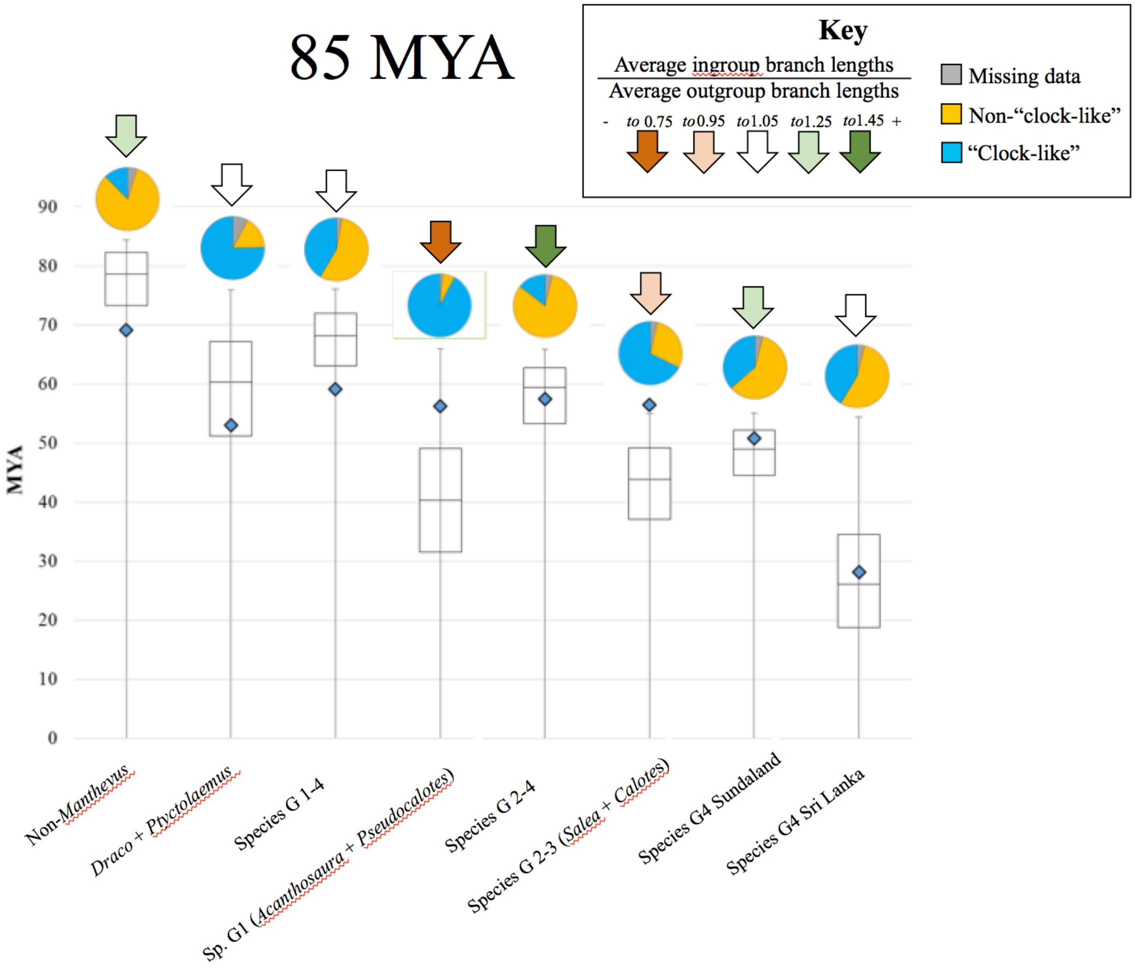

Figure 1.4 – Box-and-whisker plots, showing results of our analysis using our UCE_divergence_timing R script (minimum, 25% quartile, 75% quartile, maximum) with a minimum estimate for the age of Draconinae of 85 MYA used to calibrate the ages of the Non-*Mantheyus* clade. For subsequent subgroups, the estimated age of the clades were contained within this calibration point. For each group’s divergence timing estimate, only loci that appeared “clock-like” (ingroup age estimate did not exceed the calibration age) were used. Percentages of loci that were “clock-like” versus non-“clock-like” (likely affected by rate variation or incomplete lineage sorting), and loci with missing data for outgroups (sister species of the groups of interest) are shown in pies

above box-and-whisker plots (see key). Clades with red arrows show slow-downs relative to their outgroups i.e. average cumulative branch lengths leading to ingroup taxa from the ingroup/outgroup node are shorter than those leading to the outgroups (this appears to be correlated with underestimates of divergence times using the naïve strict clock method), clades with green arrows show rate speed-ups relative to their outgroups i.e. average cumulative branch lengths leading to ingroup taxa are longer than those leading to the outgroups. Bayesian estimates of divergences times performed in BEAST are shown as small blue diamonds, for comparison.

Chapter 2

Ghosts of islands past: *in situ* insular origins of the Indo-Himalayan Dragon lizards

Jesse L. Grismer, James A. Schulte, Philipp Wagner, and Rafe M. Brown

Abstract

Aim (1) Evaluate the origins of the endemic Indo-Himalayan Dragon lizards using new multilocus DNA sequence geological data derived from recent studies of the formation of the Himalaya and the Irrawaddy Mountain ranges. To understand the absence of widespread tropical lineages on the Indian subcontinent, we (2) assess the possible extinction of Indian Dragon lizards associated with the Holocene aridification of India following the uplift of the Himalayas.

Location Indo-Himalaya of South and Southeast Asia

Methods We estimated time calibrated phylogenies and ancestral range reconstructions to investigate the origins of Indo-Himalayan Dragon lizards. We evaluated divergence times and ancestral areas estimates in the context of two competing geological models that differ by the timing and nature of the collision of India with Eurasia. Finally, we estimated branch-specific rate shifts using a Bayesian analysis of diversification in order to test for statistically significant shifts, and declines, in lineage-specific speciation rates leading to specifically Indian and Sri Lankan Dragon lizard groups.

Results The Indo-Himalayan lineage diversified from each other approximately 53 MYA, with the Indo-Himalayan taxa appearing to have originated around 47.2 MYA, well before the India's Soft Collision with Eurasia at 30–40 MYA. Moreover, the three major lineages within the remainder of the Dragon lizard phylogeny appear to have rapidly diversified between 50.8–59.1 MYA. Lastly, we recovered the endemic Sri Lankan genera as monophyletic, initially diversifying during the Oligocene at approximately 28.1 MYA, and well supported as sister to a clade of endemic Sundaland genera.

Main conclusions The results suggest that the Indo-Himalayan lineages may have evolved on a hypothesized paleo-island arc before the Hard Collision of India with Eurasia. This island arc was eventually compressed into the various mountain ranges of the Himalayas and Irrawaddy Mountains with the uplift of Indo-Himalaya, and these Dragon lizards have maintained these historical archipelago-like, ("sky island") fragmented distributions into their modern day locations. We did not find statistical support for the Himalayas associated with the extinction of tropical Indian species. However, we provide strong circumstantial evidence of extinction as an explanation for unexpected phylogenetic relationships and the conspicuous absence of Dragon lizards in India.

Introduction

Biogeographic and macro-evolutionary hypotheses are commonly tested with phylogenetic analyses that focus on taxa distributed across a particular geographic area, geological feature, or ecological gradient (Ronquist and Sanmartin, 2011). A standard working null hypothesis for empirical studies is that evolutionary patterns and phylogenetic relationships mirror the geologic and climatic processes of a given area (Fig. 2.1A: Waters *et al*. 2001; Donoghue *et al*. 2001; Page and Charleston 1998; McDowall 1978; MacArthur 1973). This initial geographic principle is often exemplified by concepts such as "species pumps" (Haffer 2008) hypothesized to have generated diversity in well studied "sky islands" systems (Knowles 2001; Antonelli *et al*. 2009; Manthey & Moyle 2015; Papadopoulou & Knowles, 2015), or diversification "conveyor belts" such as the Hawaiian archipelago where phylogenetic relationships of organisms track the formation of the island chain (Funk & Wagner 1995; Baldwin 2007; Baldwin *et al.* 2011; Roderick *et al*. 2015).

When studies recover anomalous or topological relationships (Fig. 2.1B) that do not follow the historical geologic processes and/or climatic patterns of a given area, potential explanations can include historical events, species interactions, asymmetrical dispersal rates, or systematic error like sampling biases (Urquhart *et al.* 2009; Bilton *et al.* 2001; Samuel *et al*. 1998). In contrast, extinction is less frequently postulated (Sanmaritan and Ronquist 2004), but is commonly evoked when these anomalous relationships are inferred in taxa with widely disjunct modern distributions, such as Chinese and American Alligators, American Hellbenders and Japanese Giant Salamander, Australian and American Marsupials, and Baobab Tress in Africa, Madagascar, and Australia (Vitt and Caldwell 2013; Meredith *et al.* 2007; Sanmartin and Ronquist 2004; Baum *et al.* 1998;). Nevertheless, limited evidence suggests that it may be possible for extinction to produce non-intuitive topological relationships and faunal disjunctions across smaller geographic areas with complex geologic and climatic histories (Boutler *et* *al.* 1988; Sanmartin and Ronquist 2004). We postulate that one such region is the iconic Indian subcontinent, the island of Sri Lanka, and dramatic montane topography of the Indo-Himalayas.

The Himalaya and the Irrawaddy Mountains of Indo-Himalaya are the result of an ongoing collision between the Indian and the Eurasian tectonic plates (Aitchison *et al.* 2007). High levels of floral and faunal endemism have rendered the Himalayas of particular interest to researchers focused on testing evolutionary hypotheses related to their diversification associated with the uplift of the Indo-Himalayan Mountain ranges (Friesen *et al.* 2000; Bhattarai *et al.* 2004; Agarwal *et al.* 2014; Fumin *et al.* 2014). With advanced geological and climatological methodologies a resurgence of recent studies has reawakened biogeographers' former attention to the timing and nature of the India/Eurasia collision (Acharya *et al*. 2007; Aitchison *et al*. 2007; Ali and Aitchison, 2008; van Hinsbergen *et al*. 2011; van Hinsbergen *et al.* 2012; Ponton *et al*. 2012; Bouilhol *et al.* 2013).

From these studies two well-supported geologic models depicting the timing and formation of the Himalaya and the Irrawaddy Mountain ranges have generated clear, exclusive, and testable predictions for biogeographers. The One Collision Model (and our related One Collision Hypothesis: OCH) suggests that there were ephemeral land bridge connections with India 50–55 MYA, and a single continental collision in the early Oligocene 34 MYA resulting in the uplift of the Indo-Himalaya mountain ranges (Acton 1999; Aitchison *et al.* 2007; Ali and Aitchison 2008). In contrast, the more recent Two Collision Model (and our associated Two Collision Hypothesis: TCH) postulates that an offshore paleo-island archipelago (often referred to as the Tibetan micro-continent;

(Valdiya 2015) formed off the west coast of the Thai-Malay Peninsula, between Eurasia and the approaching Indian subcontinent (van Hinsbergen *et al.* 2012). Under the TCH, this paleo-island arc along with various marine sediments first collided with Eurasia in a 'soft collision' spanning 30–40 MYA (Bouilhol *et al.* 2013), followed by the 'hard collision' (the final continental connection) of the Indian subcontinent and Eurasia between 20–25 MYA (Fig. 2.3). Additionally, proponents of the Two Collision Model have inferred that the Tibetan micro-continent along with various geologic deposits (Acharya *et al*. 2007; Bouilhol *et al.* 2013; van Hinsbergen *et al.* 2012) was eventually folded and uplifted, into the various mountain arcs of the modern Himalaya and Irrawaddy Mountains (Fig. 2.3D). A commonly cited demonstration of this extreme case of vertical paleotransport is the observation that the peak of Mount Everest is composed of marine sediments (McPhee 1981).

Under either the OCH or TCH, recent work has demonstrated that the during the uplift of these mountain ranges, the Himalayas in particular, may have increasingly become a rain-shadow for the rest of the Indian subcontinent (Fleitmann *et al.* 2003; Fleitmann *et al.* 2007; Ponton *et al*. 2012) shifting monsoonal patterns and tropical habitat, causing an aridification during the Holocene. During this drying event associated with the continued uplift of the Himalayas, it has been hypothesized that India may have experienced a loss of tropical diversity (Ponton *et al.* 2012). This is a testable hypothesis, given a suitably distributed empirical study system and a robust phylogenetic estimate of species relationships.

Here we evaluate the major predictions of the OCH versus the TCH with regard to the formation of the Himalaya and Irrawaddy Mountains. We use new data bearing on the phylogeny of the South and Southeast Asian Dragon Lizard radiation, in an attempt to investigate the role of the paleogeographic template in generating high elevation Dragon Lizard endemism in Indo-Himalaya. We also evaluate predictions derived from the expectation that a subsequent aridification of India may have played a role in the conspicuous absence of some—and diversification of other—tropical Indian lineages.

Empirical study system

Dragon lizards from South and Southeast Asia represent an array of genera with geographically circumscribed radiations in Indo-Himalaya, Sri Lanka, India, Indochina, and Sundaland (Fig. 2.2). Their members exhibit a range of ecomorphological and habitat preferences (arid and tropical), making them an ideal system to test biogeographic hypotheses derived from predictions of the major geological reconstructions of the formation of the Himalayas and the Irrawaddy Mountains, and the subsequent aridification of India. Previous phylogenetic analyses of the Dragon lizards have not focused on lineages from Indo-Himalaya and no hypothesis has been put forth as to where and when these species originated. Earlier work on Dragon lizards has focused primarily on the origins of Indian and Mainland Asian Dragon Lizard lineages, and has demonstrated the genera from these areas to be paraphyletic (Macey *et al.* 2000; Schulet *et al.* 2004; Grismer *et al.* 2016). These authors attributed these relationships to the rapid exchange of Indian and Eurasian faunas through land bridges during the mid-to-late Eocene. Interestingly, these studies found support for reciprocal Sri Lankan and Sundaland monophyly, with divergences between these two clades estimated at approximately 50 MYA (Grismer *et al.* 2016). This split is of particular interest because at 50–55 MYA the Indian subcontinent has been postulated to been connected to Eurasia via a land bridge (Ali and Atichison 2008), which would have included Sri Lanka as it was still part of today's Western Ghats of India (Fig. 2.3), and did not separate from India until 25–20 MYA. Under this reconstruction and the assumption of faunal exchange over available land connectivity, one might expect to observe a clade of Indian taxa sister to a Sundaland or Sri Lankan clade (Fig. 2.4B), coincident with the geologic break up of these areas.

Seemingly at odds with this pure-vicariance expectation, Indian taxa have been demonstrated to have phylogenetic affinities with Mainland Asian and Sundaic genera (Grismer *et al.* 2016). Grismer *et al.* (2016) concluded that additional taxa would be needed to test hypotheses related to the origins of Indo-Himalayan and Sri Lankan genera with respect to the Sundaland genera. Here we test predictions regarding the origins of endemic Indo-Himalayan Dragon lizards with taxonomic sampling expanded beyond previous studies (Macey *et al.* 2000; Schulte *et al.* 2004; Zug *et al.* 2006; Grismer *et al.* 2016) and focusing on missing Indo-Himalayan, Sri Lankan, and Sundaland lineages. Our expanded data set includes sequence data from museum specimens of Sundaland endemic genera (*Dendrogama, Lophocalotes,* and *Harpesaurus*) that have not been collected or sampled for genetic material since their original 19th Century descriptions.

With this dataset we investigate the origins of the Indo-Himalayan Dragon lizards by comparing our recovered phylogenetic relationships to the expected topologies that could be generated under: (1) the traditional OCH (Fig. 2.5B) of a vicariant origin via the uplift of the Himalaya and Irrawaddy Mountains during the hard collision 34 MYA (Moody, 1980; Acton 1999; Macey *et al.* 2000; Schleich and Kastle, 2002; Aitchison *et al.* 2007; Ali and Aitchison 2008) and (2) a Tibetan micro-continent origin consistent with the TCH (Fig. 2.5B) as predicted from recent geologic studies (Acharya *et al*. 2007; Bouilhol *et al.* 2013; van Hinsbergen *et al.* 2012).

In addition, given the previously reported anomalous relationships between the Sundaland and Sri Lankan genera (Fig. 2.4A), and in absence of other well-supported explanations, we find the Indian Holocene aridification scenario to be the most plausible explanation for the Indian subcontinent's "missing" tropical Dragon lizards. In this study we undertook a test of this hypothesis by examining rate shifts in diversification along branches leading to Sri Lankan and Indian taxa for the signature of decreased speciation (i.e. extinction). We present a new empirical study system demonstrating how continental collision and tectonic, orogeny generated climatic shifts may have been responsible not only for generating and maintaining high levels of endemic land vertebrate diversity, but which also may have contributed to extinction of related and codistrubuted tropical taxa. Finally, with the most taxonomically comprehensive Dragon lizard clade (The Draconinae) phylogenetic dataset to date (Moody, 1980; Macey *et al.* 2000; Melville *et al.* 2009; Schulte *et al.* 2004; Zug *et al.* 2006), we summarize and synthesize our cumulative understanding of the biogeography and evolution of the entire Draconinae and emphasize the clade's rich potential for a wealth of future studies of evolutionary biology of lizards.

Materials and Methods

Genetic and taxonomic sampling

We compiled a new dataset composed of DNA sequences from all available previously sampled Dragon Lizard taxa (species, genera), augmented with new data from our own collections and those of our collaborators (Macey et al. 2000; Schulte *et al.* 2004; Zug *et al.* 2006; Melvile *et al.* 2009; Grismer *et al.* 2016). In total, the dataset included 21 of the 25 Dragon Lizard genera representing 53 individuals (Appendix 3), including all but one of the Indo-Himalayan genera (*Oriocalotes*), and three genera from Sundaland (*Complictus, Hypsocalotes,* and *Pseudocophotis*). This data set includes novel taxon sampling in the form of new sequence data, collected from genetic tissue samples corresponding to museum specimens of Sundaland endemic genera, notably *Dendragama boulengeri* (ZFMK 50532)*, Lophocalotes ludekingi* (ZFMK 46706), and *Harpesaurus beccarii* (ZFMK 48896). We included genera from the remaining Dragon Lizard subfamilies, *Physignathus* and *Laudakia* as outgroups. Genomic DNA was extracted from muscle or liver tissue samples using a DNeasy tissue kit (Qiagen, Inc.) and sequenced for the mitochondrial and nuclear genes, ND2 (primers from Macy *et al.* 2000) and RAG-1 (primers from Melville *et al.* 2009), respectively, using standard PCR and Sanger sequencing protocols as described in Grismer *et al.* (2016). We edited the sequences and aligned them within Geneious Pro 5.0.4 (http://www.geneious.com, Kearse *et al.* 2012) and these new sequence data were combined with existing data from Grismer *et al.* (2016). We selected ND2 and RAG-1 because they are the most frequently sequenced markers across acrodont lizards and therefore provide maximum taxonomic coverage keeping our datasets consistent with previous studies (Macey *et al.* 2000; Schulte *et al.* 2004; Zug *et al.* 2006; Melville *et al.* 2009; Grismer *et al.* 2016).

Phylogenetic, biogeographic, and speciation rate analyses

Using 85 MYA as a minimum age limit for the ancestor of the Draconinae (Grismer *et al.* 2016), divergence dates for subclades were estimated with Bayesian analyses in the program BEAST 2.3(Bouckaert *et al.* 2014) using the ND2 and RAG-1 datasets with linked clock and tree models. Substitution models and partitions for each dataset were estimated using jModelTest 2.0 (Guindon and Gascuel 2003; Darriba *et al.* 2012) and separate nucleotide substitution models were used for each gene, and due to the risk of substitution saturation, we performed analyses including and excluding the third codon position for the ND2 alignment but not for the RAG-1 data. We applied Birth-Death tree priors and constrained three nodes (pink circles: Fig. 2.6) to match the relationships resolved using with a novel dataset of 4747 nuclear loci in Grismer *et al.* (2016), and estimated the remaining relationships. We used a relaxed uncorrelated lognormal clock model and an exponential prior for the mean rate of each partition. Default values were used for all other priors, and the analyses were ran for 150 million generations sampling every 12,000 generations, with stationarity assessed in Tracer 1.6 (Rambaut *et al.* 2014). The first 25% of trees were discarded as burn-in and the maximum clade credibility tree with median node heights was summarized using TreeAnnotator 2.3 (Bouckaert *et al.* 2014).

We performed Bayesian ancestral-area estimations across the phylogeny of Dragon lizards using the R package ‘BioGeoBears’ (Matzke, 2013a). BioGeoBears calculates maximum-likelihood estimates of the ancestral states at internal nodes by modeling transitions between geographical ranges along phylogenetic branches as a function of time. Because different ancestral-area reconstructions are based on different assumptions and can produce conflicting results (Matzke, 2013a, 2013b), we compared the dispersal–extinction–cladogenesis model (DEC), a likelihood implementation of dispersal–vicariance analysis (DIVALIKE), and the range-evolution model of the

Bayesian binary (BAYAREALIKE) models. We also included a comparison of all models with and without the founder-event speciation parameter (+J), which can be added to any of these previously described models (Matzke, 2013b; and references therein) as a parameter to model descendant lineages colonizing a new area not inhabited by ancestral, parental types. We used the Akaike information criterion (AIC) to compare model fit (Matzke, 2013a; 2013b).

We estimated branch-specific rates of speciation using a Bayesian analysis of rates of diversification using the R package 'BAMM' 2.3.0 (Rabosky *et al.* 2014) to test for rate shifts leading to Indian and Sri Lankan lineages in our Dragon Lizard phylogeny. The general model assumes that phylogenetic trees may have been shaped by a heterogeneous mixture of distinct evolutionary regimes of speciation and extinction and BAMM enables reconstruction of marginal posterior distributions of speciation and extinction rates on individual branches of a reconstructed phylogenetic tree (Rabosky 2014). We accounted for incomplete taxon sampling directly in the BAMM model itself and used 'BAMMtools' for estimating priors, to perform the analysis, and to visualize results (Rabosky 2014; Rabosky *et al.* 2014). To ensure that the estimates of rate shifts at shallower areas of the tree were not being influenced by older more divergent lineages, we preformed one analysis including the outgroup and a highly divergent draconinae stem lineage (*Mantheyus*), and another excluding this genus and the outgroups.

Results

The results from our phylogenetic analyses placed the Indochinese endemic *Mantheyus* as the sister lineage to all the remaining agamid lineages in the phylogeny.

The next lineage to diverge was a clade containing *Draco*, sister to the monophyletic Indo-Himalayan lineages. *Draco* and the Indo-Himalayan lineages split from one another approximately 53.0 ± 8 MYA and are sister to the remaining members of the subfamily (Fig. 2.6). The Indo-Himalayan clade appears to have originated at 47.2 ± 4 MYA, well before the beginning of the soft collision temporal framework (Fig. 2.6). The three major lineages within the remainder of the subfamily appear to have rapidly diversified between 50.8–59.1 MYA (Fig. 2.6), which encompasses the historically problematic section of the Dragon Lizard phylogeny (pink circles: Fig. 2.6), that were just recently resolved through the use of 4536 nuclear loci derived from ultraconserved elements (Grismer *et al.* 2016). In contrast to Grismer *et al.* (2016), we recovered a polyphyletic *Japalura* with *J. planidorsata* sister to *Calotes, J. variegata* as sister to *Oriotaris*, and the Indochinese *Japalura* species sister to *Pseudocalotes*; all with high support. The three rare Sumatran endemics from Sundaland (*Dendragama, Lophocalotes,* and *Harpesaurus*), genetically sampled here for the first time, were found to be composed of a monophyletic group that diverged from a clade comprised of *Aphaniotis, Bronchocela,* and *Gonocephalus* 46.2 ± 4 MYA. This clade of Sundaland species formed the lineage sister to a Sri Lankan clade comprised of *Lyriocephalus, Cophotis,* and *Ceratophora*. The two aforementioned major clades diverged from on another approximately 50.8 ± 7 MYA (clade A: 6). Lastly, we recovered the endemic Sri Lankan genera as monophyletic, initially diversifying during the Oligocene at 28.1 ± 3 MYA.

For the ancestral-area estimations BAYAREALIKE + J was selected as the best-fit model. Along with the relationships and divergence times from the BEAST analysis, our biogeoraphic reconstructions were consistent with the findings of Grismer *et al.*

(2016). The BioGeoBears and BEAST analyses both indicated that Dragon Lizard lineages originated from a mainland Asian ancestor 92 ± 10 MYA (Fig. 2.6). A continental Asian ancestor was the most likely state for all major clades with the exception of Clade A (Fig. 2.6). The ancestor of the combined Sundaland/Sri Lanka clade (Clade A: Fig. 2.6) was either of Sundaic or Sri Lankan in origin. Lastly, our analysis inferred that all Indian lineages descended from mainland Asian ancestors during the Eocene (Fig. 2.6) consistent with the findings of Grismer *et al.* (2016). The results from our BAMM analyses returned similar estimates rates of speciation shifts across our Dragon Lizard phylogeny (Appendix 4). Both the full analysis and analysis of excluded lineages identified three topological regions of rate-shifts, one of which was statistically significant. The only rate shift of marginal significance was an initial increase in speciation (74% probability) along the branch leading to all lineages in our phylogeny, excluding *Mantheyus* (Appendix 4).

Discussion

Origins of the Indo-Himalayan lineages

Our results allow us to reject a vicariant hard collision origin for the Indo-Himalayan Dragon lizards under the One Collision Hypothesis (OCH). Rejecting the predictions of the OCH in favor of the Two Collision Hypothesis (TCH), strongly implies that Dragon Lizard lineages may have originated on a paleo-island archipelago 47.2–53 MYA before the Soft and Hard Collisions of Indian with Eurasia 20–40 MYA. Ali and Aitchison, (2008), postulated India was first connected to Eurasia via land bridges between 50–55 MYA (Fig. 2.3A), and we hypothesize that during this time the position of this paleo-island arc and the resulting reduction of distance from the continental shelf caused by the advancing India subcontinent, may have allowed for overwater colonization (or through an ephemeral land bridge) by continental Asian lineages (Figs. 3, 6). The paleo-island arc origin hypothesis for the Indo-Himalayan lineages is supported by several additional lines of evidence. First, the current, fragmented distribution of these lineages across the various mountain arcs in Indo-Himalaya, is consistent with an interpretation of an *in situ* island radiation—and it mirrors the order and scale (relative size of paleo-island landmasses), inferred from geological formations (Fig. 2.3A–D, 5A); Bouilhol *et al.* 2013; van Hinsbergen *et al.* 2012). Maintaining paleo-island distributions in Dragon lizards also is seen in species of the Flying Dragon, genus *Draco* on the island of Sulawesi in Indonesia (McGuire *et al.* 2007), where endemic species of *Draco* are confined to areas that used to be isolated islands when sea levels were lower. Lastly, support of this biogeographic scenario is our inference of the systematic position of *Japalura variegata*: more closely related to the other Indo-Himalayan (non-*Japalura*) lineages to the exclusion of other traditionally recognized species of *Japalura*. This finding reinforces our confidence in the interpretation that all Indo-Himalayan lineages share a single origin, and that these lineages may have all originated on Tibetan micro-continent paleo-island arc. Finally, support for the paleo-island origin scenario is derived from comparisons of biogeographic patterns with unrelated taxa, which may have diversified with similar patterns as a result of their being subject to the same geological mechanisms. The timing of the TCH also is supported by the estimated age of the ancestor for Indo-Himalayan endemic Bent-toed geckos (genus *Cyrtodactylus*), at approximately 51 MYA, well before the Soft Collision with Eurasia at 30–40 MYA

(Agarwal *et al.* 2014). This clade of Himalayan Gecko is sister to the remaining clades of South and Southeast Asian species*,* mirroring the pattern of relationships inferred here for Indo-Himalayan Dragon lizards. Although Agarwal *et al.* (2014) did not invoke a paleo-island origin for Indo-Himalayan Bent-toed geckos, they did not evaluate their findings in context of the geological models discussed here. Nevertheless, their findings corroborate ours in support for a new biogeographic scenario, which is most consistent with the TCH proposed by (Bouilhol *et al.* 2013; see also van Hinsbergen *et al.* 2012; Acharaya *et al.* 2007). This scenario should be more intensively evaluated with renewed attention to the phylogenetic relationships, explicit predictions derived from them, and timing of diversification in additional, unrelated, Indo-Himalayan endemic flora and fauna.

The origin of the Flying Dragons, genus *Draco*, presents an interesting biogeographic question. *Draco* is the sister group to all the Indo-Himalayan lineages (Fig. 2.6) and has extensive radiations in Indochina, Sundaland, the Philippines, and Wallacea (Manthey and Grossmann, 1997; McGuire and Alcala, 2000; McGuire *et al.* 2007; Manthey, 2008). However, only three species have been documented in India (one of which is endemic), but an unidentified specimen from the Jorpokhari and Pachthar districts in Nepal has been reported (Schleich and Kastle, 2009). Given *Draco*'s modern-day distribution, its phylogenetic position evokes the additional question of whether the clade originated in Asia, India, or on the paleo-island arc with Indo-Himalayan lineages. Our results are most consistent with a southern Indochina origin (25.7 ± 3 MYA), followed by subsequent range expansion throughout Southeast Asia through southern Indochina and Sundaland following the invasion of dipterocarp forests from India during its initial connections with Eurasia and during the Hard Collision (Dayanandan *et al.*

1999). In our biogeographic analyses we did not code *Draco* as a primarily Indian or Indo-Himalayan lineage because; (1) *D. maculatus* and *D. norvilli* have limited distributions in the Assam area of northeastern India (a geological transition area between Indochina and Indo-Himalaya); and (2) *D. dussumieri*, a taxon sister to Sundaland species, is clearly the result of a minor, single-leaf colonization from mainland Asia (Honda *et al*. 1999). McGuire *et al.* (2001) demonstrated that *D. maculatus* is member of an Indochinese/Sundaland radiation, presumably having colonized the Assam region (N.E. India) from the east. However, inclusion of Indian species and resolving the Indo-Himalayan *Draco* records*,* and properly coding their distributions in future studies, will be vital into an eventual, refined, understanding the evolutionary origins of flying lizards and possibly the Indo-Himalayan lineages.

Although several phylogenetic studies of Indian and Southeast Asia taxa have focused on the traditional OCH interpretation, these studies were focused mostly on the origins of India taxa with respect to Southeast Asian species (Dayanandan *et al.* 1999; Bossuyt and Milinkovitch 2001; Gower *et al.* 2002). Although these studies demonstrated that many Indian groups originated well before, or during the time of the hard collision (25–35 MYA), many studies thus far have lacked the relevant sampling of Indo-Himalayan taxa for testing predictions of the TCH scenario (van der Meijden *et al.* 2007; Macey *et al*. 2008; Klaus *et al*. 2010). Identifying Indo-Himalayan clades that include endemic species, and targeting these for phylogeny-based biogeographic inference, is a priority for future studies aimed at refining our understanding of the evolutionary response of the region's biota to its complex geological history.

India's missing agamid lizards

Biogeographers assume that extinction has both episodically and continuously affected the outcome of diversification while, simultaneously impacting the distributions of extant lineages (Brown and Kodric-Brown 1977; Boutler *et* al. 1988; Pimm and Raven 2000). In many celebrated examples, the explanation for today's presence and even dominance of particular taxa on a particular landmass (e.g., marsupials of Australia) can clearly be attributed to historical events, contingency, absence or presence of competitors and/or predators, or formidable periods of isolation (Raup 1991). However, how do we explain the absence of a group in an area, or from a landmass, where all empirical evidence and theoretical considerations would predict it should be present? Asking why a group may be *absent* from a particular biogeographic region (e.g., the absence of caecilian amphibians on Madagascar) can be just as conceptual, thought provoking, and occasionally informative, as investigating the evolutionary and geographical possibilities underlying a lineage's *presence* on a particular landmass (Lomolino *et al.* 2010; Mcarthy 2011). However, negative data are seldom conducive to statistical tests of biogeographical hypotheses (Darlington 1965; Stevens 1989; Rohde 1992; de Queiroz 2014). As such, with the field's increasing expectation of a rigorous, hypothesis testing framework for biogeographical inference, the curious absence of particular taxa, where we might expect them to be present, seems to be often overlooked.

The conspicuous near-absence of co-distributed Dragon lizards with distributions spanning India and Sri Lanka (notable exceptions include *Sitana* and *Otocryptis*), and the anomalous absences of clades with India+Sri Lanka sister relationships begs for a causal explanation. In this case, the observation points to one possible explanation that might involve extinction of tropical Indian species which might have been a result of the aridification of India (Fleitmann *et al.* 2003; Fleitmann *et al.* 2007; Ponton *et al.* 2012). To better illustrate this possible explanation for a conspicuous absence of expected Dragon Lizard lineages, we will first discuss an example involving the endemic Indian and Sir Lankan genera, *Sitana* and *Otocryptis* (Node B: Fig. 2.6), which illustrates the expected relationship (Fig. 2.4B) following climatic shifts and the geologic break up these areas (Ali and Aitchison, 2008; van Hinsbergen *et al.* 2011; van Hinsbergen *et al.* 2012). In our phylogeny *Sitana* and *Otocryptis* are sister to the Mainland Asian genera, *Japalura* and *Pseudocalotes* and most likely originated in Mainland Asia at approximately 48 ± 3 MYA (Node B: Fig. 2.6)*,* when India and Sri Lanka were connected to Eurasia by the Eocene land bridges (Aitchison *et al.* 2007; Ali and Aitchison, 2008; Grismer *et al.* 2016). The genus *Otocryptis* currently is restricted to tropical Sri Lanka (with the exception of one tropical Indian species) and *Sitana* is restricted to arid sub-tropics of Peninsular India and Sri Lanka. A divergence of 27 ± 4 MYA between *Sitana* and *Otocryptis* is consentient with the timing of and Oligocene break up of India and Sri Lanka, and this sister relationship of arid-adapted and tropical lineages would be expected with India’s Holocene aridifcation (Fleitmann *et al.* 2003; Fleitmann *et al.* 2007; Ponton *et al.* 2012). The inferred sister relationships, temporal framework, and today’s distributions of these genera are consistent with the known geology of the region (Fig. 2.4B).

However, the split of the other Sri Lankan genera form the Sundalaic linages at approximately 50.8 ± 7 MYA (Node A: Fig. 2.6) is inconsistent with the geology of the region because Sri Lanka was continuous with the Western Ghats until the early Oligocene (Valdiya 2015). Additionally, the inferred ancestor at Node B (Fig. 2.6) suggests during this time the tropical Indian subcontinent was independently colonized by the ancestors of *Salea, Sitana*, and *Otocryptis* (Fig. 2.6). Given this finding, the absence of Indian Dragon Lizard clade (tropical or arid) sister to the Sri Lankan genera (Node B: Fig. 2.6) the possibility of regional extinction of tropical Indian lineages may be plausible. Despite no statistical support for a decrease in speciation rates along branches leading to Indian and Sri Lankan genera (Appendix 4), our analyses support the interpretation of increased speciation rates during the Eocene floral and faunal Exchange between the Indian subcontinent and Eurasia (Grismer *et al.* 2016). Although the speciation + extinction analyses are uninformative with regard to our extinction hypothesis, the geological, phylogenetic, and biogeographical data indicate that suitable tropical habitat was "available" for Dragon Lizard lineages to exploit in India 45–55 MYA. The conspicuous absence of contemporary Indian species (especially the absence of tropical species) currently lacks an alternative explanation and, as such, aridification-associated extinction remains a reasonable working hypothesis

Our study demonstrates how anomalous empirical phylogenetic patterns associated with major geologic rearrangements and climatic shifts, can be fertile grounds for generating alternative, plausible working biogeographic hypotheses. Future studies could benefit from the inclusion of extinction as a possible causal mechanism, even in the absence of strong statistical support, or in the case of smaller scale phylogenetic problems, not amenable to macroevolutionary hypothesis testing.

Broader patterns in dragon lizard biogeography

We present the most comprehensive phylogenetic study of the South and Southeast Dragon lizards to date. The results of our phylogenetic and rate shift analyses characterize a general pattern of diversification in which deeper divergences are associated with the India–Eurasia land-bridge connections (Node A–D), and more recent diversifications could be associated with the hard collision 20–25 MYA (Fig. 2.6). Today, mainland Asian lineages are represented by two clades (The *Acanthosaura* and *Calotes* groups: Fig. 2.6; Nodes C, D). Our ancestral area estimates infer that all contained genera (except *Japalura planidorsata*) descended from mainland Asian ancestors 34.3–56.4 MYA, before or at the start of both soft and hard collisions (30 MYA and 20 MYA, respectively). Within the *Acanthosaura* group (Node D: Fig. 2.6), three genera (*Acanthosaura, Japalura,* and *Pseudocalotes*) contain most of the species diversity, and all may have diversified concomitantly with soft and hard collisions (18.4–37.4 MYA; Fig. 2.6). A similar pattern is observed in the *Calotes* Group (Node C: Fig. 2.6), in which diversification began at approximately 34.3 ± 4 MYA, involving a split between Sri Lankan and the mainland Asian lineages at during the Miocene (17.1–20.6 MYA).

Our inclusion of previously unsampled Sundaland genera, *Harpesaurus, Dendragama,* and *Lophocalotes* are consistent with findings from Macey *et al.* (2000) and Grismer *et al.* (2016) in that the Sundaland endemic genera are monophyletic (with the exception of *Phoxophrys*), representing a single colonization of Sundaland approximately 46.2 ± 4 MYA (Node E: Fig. 2.6). The genera *Harpesaurus, Dendragama,* and *Lophocalotes* form a monophyletic group (Fig. 2.6), endemic to Sumatra and sister to the remaining endemic Sundaland lineages. Divergences between Sundaland genera (~7–

12, at million year intervals) appear to correspond to the episodic sea level fluctuations in Sundaland (Voris *et al.* 2000; Woodruff *et al.* 2010), which were associated with ephemeral land connections in Southeast Asia over the last 30 million years. Because Sundaland genera all contain endemic Sumatran lineages, it is possible that Sumatra served as a center of origin, allowing for the seeding of adjacent landmasses with fluctuating sea level fluctuations. Studies of patterns of diversification within Sundalaic genera *Gonocephalus* and *Bronchocela* may be informative with respect to this biogeographic possibility.

In this study we found support for the timing and formation of Indo-Himalayan mountain ranges, under the TCH for the origin ancient resident Dragon lizards lineages. We discuss one scenario for a role of extinction in the anomalous distribution of lineages in South and Southeast Asia. We anticipate that as data from other Indo-Himalayan groups become available, paleo-island arc origins (prior to the India–Eurasia collision) explanations, will be more readily considered in unrelated, codistributed groups (e.g., Agarwal *et* al. 2014). The combined approach of evaluating densely sampled empirical phylogenies in the context of greatly refined geological models provides compelling opportunities for biogeographic hypothesis testing in the celebrated but understudied Himalayan landscape.

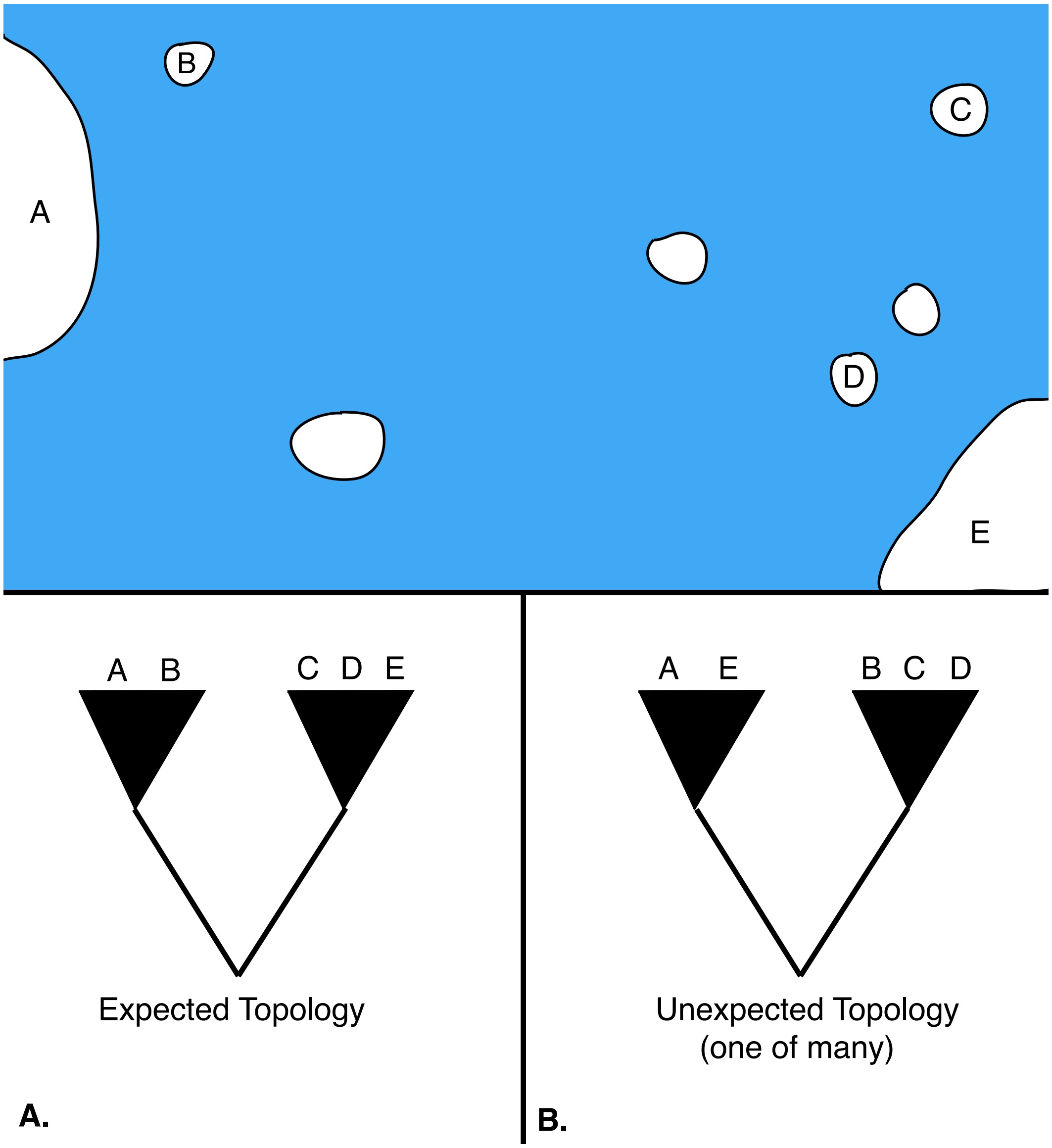

Figure 2.1– The top panel depicts a hypothetical island archipelago that was formed following a rise in sea level, and the distribution of species A–E across the resulting islands. (A) An expected topology for the relationships of species A–E, given their modern day distributions and the geologic history of the archipelago. (B) An anaomolous phylogeny, given their modern day distributions and the geologic history of the archipelago.

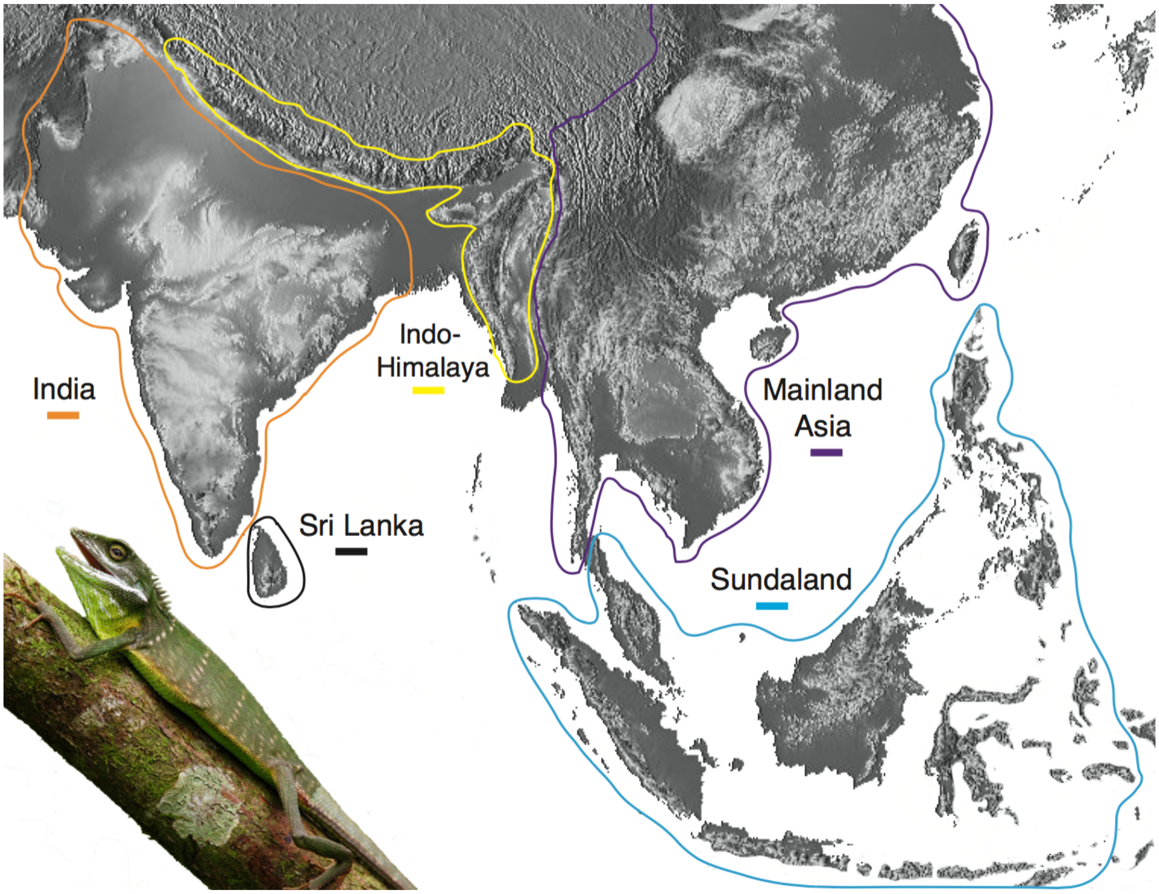

Figure 2.2– Map showing the distribution of South and Southeast Asian Dragon Lizards and their biogeographic areas. The Indo-Himalaya region includes both the Himalaya and Irrawaddy mountain ranges.

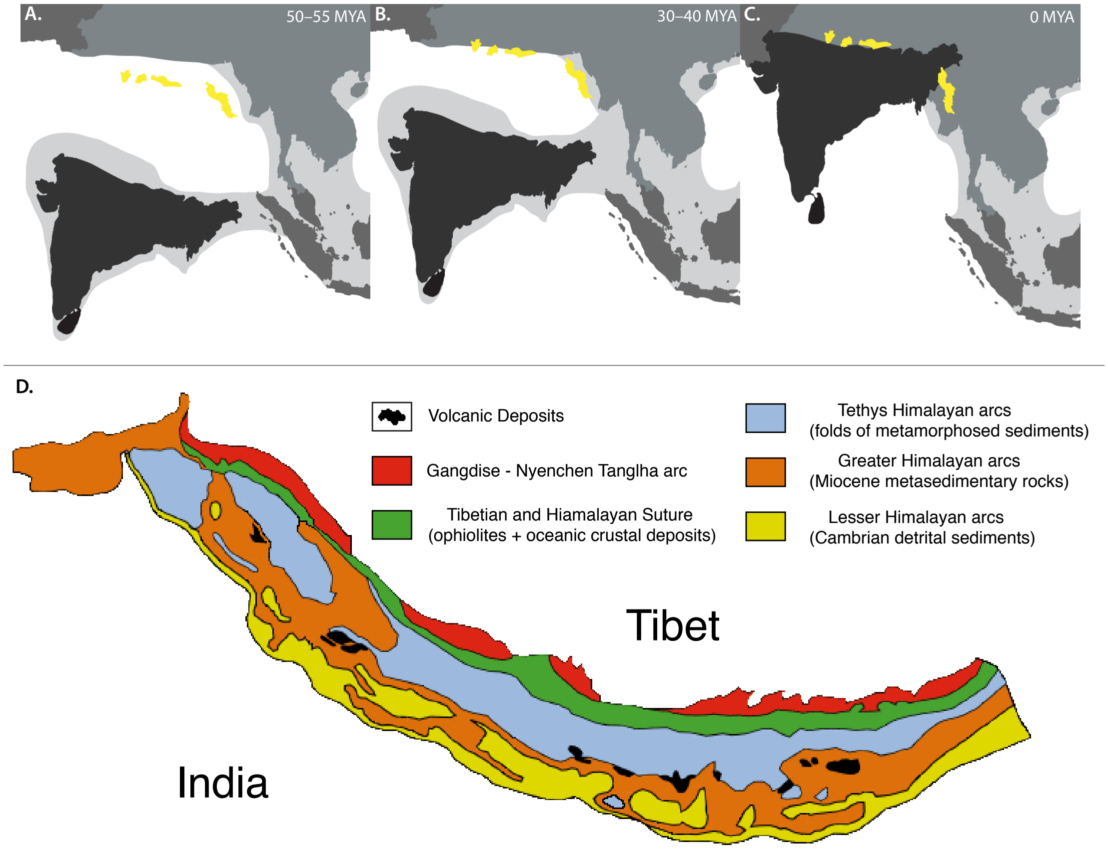

Figure 2.3– Panels depicting the TCH. A–C depict the relative position of the Indian subcontinent (including Sri Lanka) and the paleo-island arc at various time periods, with B representing the Soft Collision. (D) An enlargement of the Himalayas from panel C, showing how the amalgamation of geologic deposits India accumulated as it advanced northward was eventually folded into the various mountain arcs of the Himalayas, including oceanic crustal deposits shown in green that compose the top of Mount Everest (modified from Dèzes, 1999).

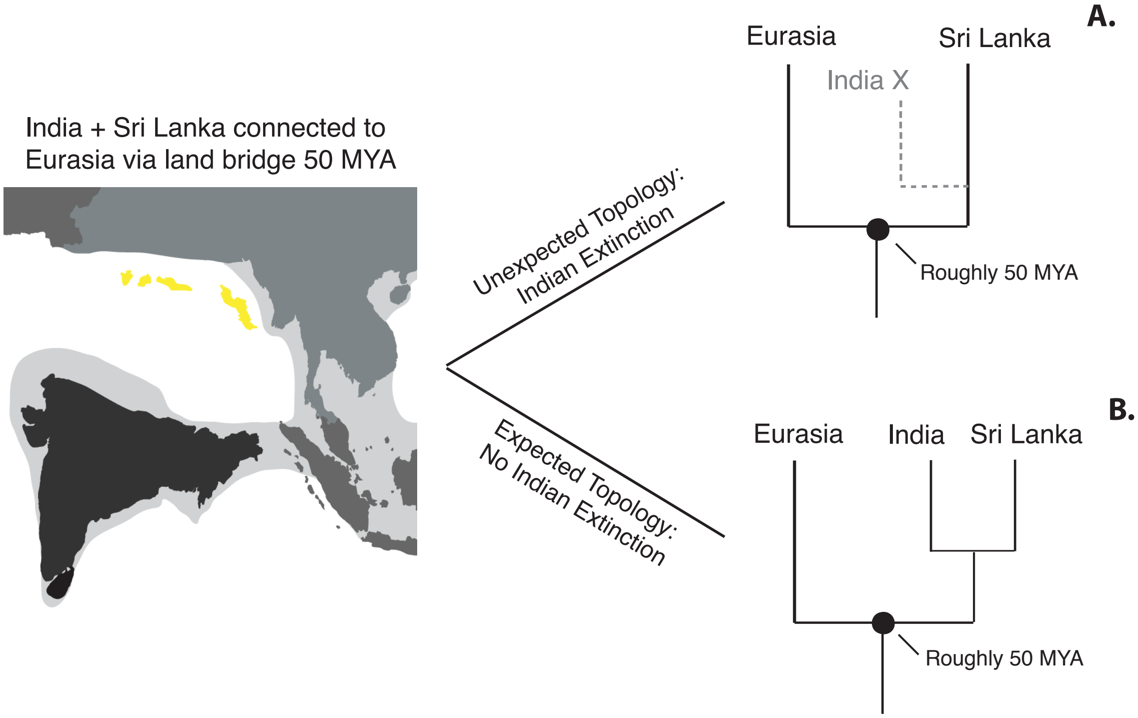

Figure 2.4– Map detailing the positions of India, Sri Lanka, the paleo-island arc, and the Eocene land bridges at 50 MYA. (A) A topology that is the *unexpected* relationships recovered by previous studies, which may be a result of extinction of Indian lineages. (B) A topology that represents *expected* relationships that follow the break up of these areas after this time period (Sri Lanka + India broke off from Eurasia then India and Sri Lanka separated ~25 MYA) and do not follow the pattern of climate-related Indian extinction.

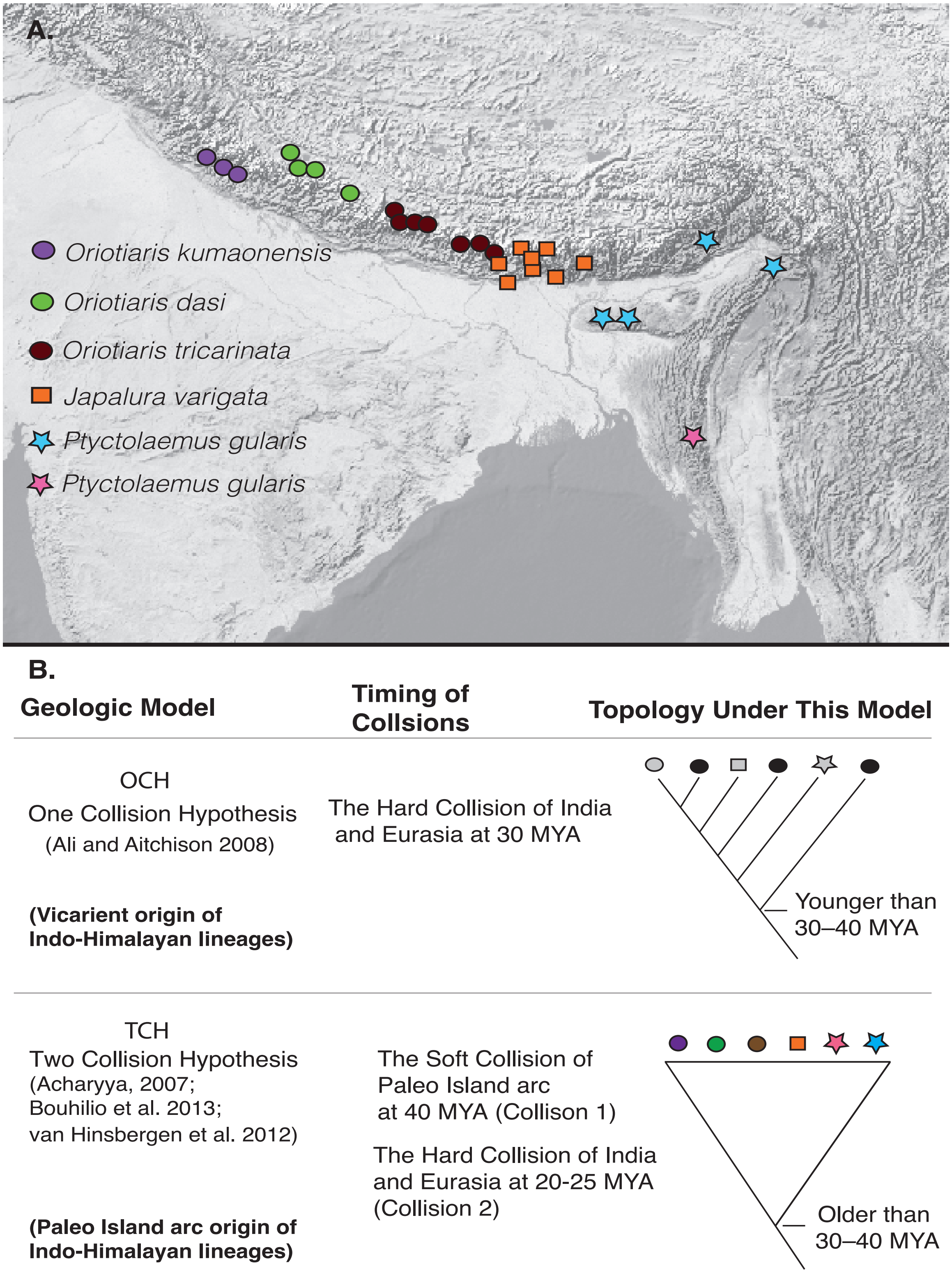

Figure 2.5– (A) A distribution map of Indo-Himalayan lineages. (B) The top panel details the estimated divergence times and topology we would expect to recover under

the OCH and the bottom panel is the estimated divergence times and relationships we would expect to recover under the TCH.

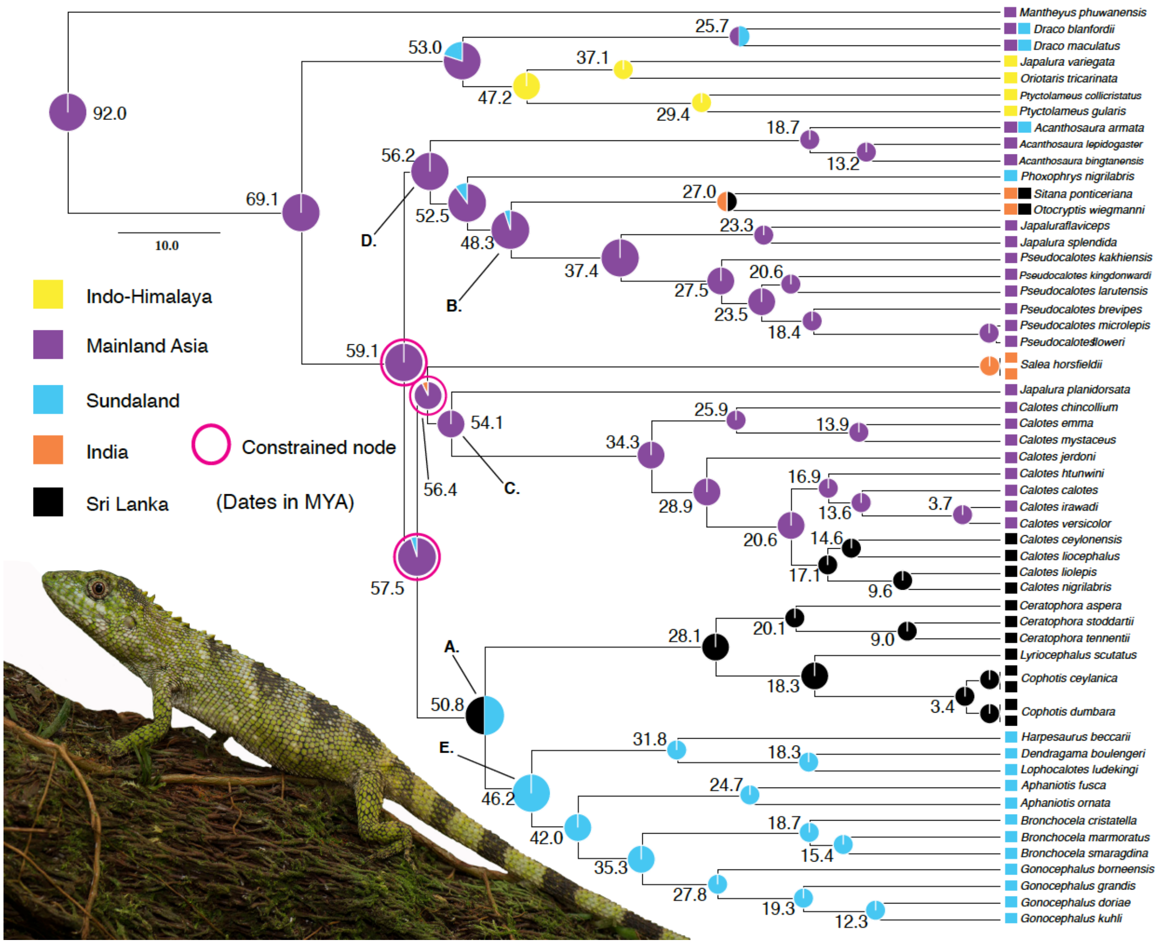

Figure 2.6– Time-calibrated phylogeny of ND2 and RAG-1 data, with black dots representing posterior probabilities higher than 0.95 followed by the estimated divergence time for that node in MYA. Colored nodes represent the ancestral ranges corresponding to Figure 2, and the pink circles represent nodes where topology was constrained based on the results of Grismer *et al*. (2016).

Chapter 3

Estimating the Dragon Lizard Tree of Life: Reevaluation of Trans-Tethys Migrations with a Fossil-Calibrated Phylogeny of the Acrodonta

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Abstract

From the Devonian until the Cretaceous, a series of island micro-continents and other geologic landmasses rifted off of Gondwanaland and rafted across the Tethys Sea. These landmasses eventually collided in succession with Laurasia forming the geologic basis for modern-day Indochina and Sundaland. Previous studies on Asian and Australian insects, fish, reptiles, birds, and mammals species have hypothesized a vicariant Gondwanan origin that for these groups, and that they would have rafted across the Tethys Sea on these micro-continental blocks. In this study we re-evaluate these Gondwana vicariant origins and Tethys Sea migrations in all lineages of Dragon Lizards. We used the most taxonomic comprehensive sampling for any study to date on Dragon Lizards using 307 species and 48 genera for two mitochondrial and nuclear loci representing the first estimate of and Agamidae Tree of Life. Additionally, we used 13 acrodont fossils with published calibration points to estimate divergence times across the Dragon Lizard lineages. We used the resulting phylogenies to estimate ancestral areas for all nodes in our phylogenies to test the null hypothesis of a Gondwana origin for Dragon Lizards from previous studies. Lastly, we used Maximum Likelihood and Bayesian analyses to reconstruct the ancestral condition for environmental preference and statistically defined morphological groups from a previous study to investigate additional support from the natural histories of the species involved in this study. Our results reject a hypothesis of a Gondwanan origin for all Dragon Lizards and are consistent with an origin in Laurasia. Furthermore, this study indicates that there were three independent dispersal events out of Laurasia into Gondwanaland represented by *Hydrosaurus*, *Draco,* and accounts for the split between *Physignathus* and the Australian/Wallacean lineages. Given the results of the ancestral reconstructions of natural histories, we hypothesize that the dispersal events of the ancestors *Hydrosaurus* and *Physignathus* were made by a large bodied, tropical, semi-aquatic, riparian specialist that may have used these micro-continental blocks migrating across the Tethys Sea, as stepping stones to disperse into Gondwana. We believe that previously held biogeographic hypotheses tend to get perpetuated rather than re-evaluated and as datasets are expanded through taxonomic and genetic sampling, and reanalyzed with modern model based analyses, we may start to see new hypotheses that invigorate new discussions on older biogeographic questions.

Introduction

Modern day East and Southeast Asia formed from a series of complex amalgamations of island micro-continental blocks, volcanic arcs, and ophiolites. From the Devonian until the Cretaceous, these landmasses rifted off Gondwanaland and were plaeotransported across the Tethys Sea (Metcalf 1988, 1996, 1999, 2011; Hall 1996; Richter and Fuller 1996), eventually colliding in succession with Laurasia (Metcalf 2013). A number of recent studies have postulated the Tethys Sea has been separated over the past 400 MYA, into three distinct intervening smaller Tethyan Sea basins (Palaeo-Tethys, Meso-Tethys, and Ceno-Tethys) by these micro-continental blocks

(Metcalf 1999, 2011). Such movements of continental fragments may have created an effective "conveyor belt" of micro-continents carrying subsets of southern hemisphere faunas from Gondwana northward to Laurasia (Macey et al. 2000 and references therein). Phylogenetic studies involving insects, fish, reptiles, birds, and mammals have hypothesized phylogenetic that the division of biotas on either sides of Wallace's Line, are possibly due to this early fragmentation of Gondwana with subsequent vicariant speciation (DeBoer and Duffels 1996; Macey et al. 1997; Keogh 1998; Metcalf et al. 2001), reinforced by the deep oceanic trench separating their modern-day distributions (Barker et al. 2002; Schulte et al. 2003).

Large-scale molecular studies on acrodont and varaniod lizards were the first to statistically address this hypothesized mechanism of primary divergence using parsimony based biogeographic methods and time-calibrated phylogenies (Macey et al. 1997, 2000; Schulte et al. 2003); these studies attempted to assess whether divergence between lineages on either side of Wallace's Line corresponded to geologic timing of micro-continents fragmentation (Macey et al. 1997, 2000; Schulte et al. 2003). With apparent similarities to previous studies involving a multitude of taxa, the authors concluded widespread faunistic vicariant origins in Gondwana across the Tethys Sea (Macey et al. 2000; Schulte et al 2003). Macey et al. (2000) reasoned that because all acrodont lizard families (Chamaeleonidae, Leiolepidae, and Agamidae) are located today on landmasses of Gondwana origin, and are separated by deep genetic divergences from their Laurasian relatives, all necessarily had phylogenetic origins rooted in Gondwanan vicariance. However, Macey et al. (2000) did not exclude the possibility of colonization from Laurasia and added that additional work was needed to fully resolve this issue, in particular, with regards to the unresolved root of the Agamidae. This would include focusing on the placement of systematically contentious and enigmatic agamid taxa (e.g. Sail Fin Lizards of the genus *Hydrosaurus*). As highlighted by Macey et al. (2000), acrodont lizards are an ideal group to revisit hypotheses concerning putative Gondwanan vicariant origins as taxonomic sampling, and gene sampling improved—and novel, model-based methods of phylogenetic analysis and biogeographic inference have become available.

Acrodont lizards are composed of Chameleons (Chamaeleonidae), Butterfly Lizards (Leiolepidae), and Dragon lizards (Agamidae; Moody 1980) that have specific radiations in Africa, Madagascar, Southwestern Asia, Central Asia, Southeast Asia, Wallacea, and Australia (Fig. 3.1). Acrodont lizards exhibit a wide range of ecological lifestyles, across a variety of habitats in tropical, temperate, and arid environments. Approximately 90% of Acrodont ecological and taxonomic diversity is contained within the Agamidae, which consists of approximately 53 genera and ~440 species. Despite this range of diversity, comprehensive studies of phylogenetic relationships within Agamidae has only been attempted twice (Moody 1980; Macey et al. 2000) and both studies presented results that supported the hypothesis of Gondwanan vicariance, leading to the origin of Acrodonta and including all its families. The study of Macey et al. (2000) included the first phylogenetic study of molecular data and provided deep insights into this diverse assemblage of species. Macey et al. (2000) used parsimony based biogeographic analyses to support a conclusion of a Gondwanan origin, via the process of vicariance, as hypothesized by Moody (1980). In support of their conclusions Moody

(1980) and Macey et al. (2000) cited the fact that the species used in their study occur on modern landmasses that have Gondwanan geologic origins.

Macey et al. (2000) also presented data that suggested the modern-day distribution of Dragon Lizards on Laurasian landmasses (Central Asia, Indochina, South Asia, and Southeast Asia) are due to dispersal from East and Southeast Asia through island micro-continental accumulations of Gondwanan origins. The authors hypothesized that taxa possibly rafted on these micro-continents, and may have had vicariant origins, and have deep genetic divergences separating them from closely related sister-group taxa in adjacent regions of Asia (Fig 2A). However, in a more recent study using genomic data Girsmer et al. (2016) investigated a subset of Macey et al. (2000)'s prediction of Gondwanan origins for all Dragon Lizards and reevaluated the hypothesis of an Indian (Gondwanan) origin. This study concluded that the Draconinae may not have had the Gondwanan origin as inferred by Macey et al. (2000) but instead may have originated in Laurasia, invading India through land bridges, prior to its hard collision with Laurasia. This study (Girsmer et al. 2016) demonstrated how expansion of taxon sampling, together with novel genetic data can provide new insights into classic, long-held biogeographical interpretations associated with diversification in a particularly diverse modern-day lineage.

In this study, we begin our reconsideration of Dragon Lizard biogeography by concurring with Macey et al. (2000)'s basic assumption that taxa with a vicariant Gondwanan origins should be deeply diverged from closely related groups in Asia. However, depart from their reasoning with Macey et al.'s (2000) assertion that a sister relationship between Asian groups and groups that originated on Gondwanan landmasses can be taken as support for a invasion from Gondwana to Laurasia (Fig. 3.2B). This is because we cannot systematically determine the polarity of dispersal from these relationships and the ancestral area of two reciprocal clades. To do so would require statistical evaluation within the context of the entire Agamidae and the encompassing Acrodonta. If the node in question were estimated to be historically Laurasian, then such an inference would contradict the previously held vicariant Gondwanan origins hypothesis (Macey et al. 2000; Schulte et al. 2003) and would introduce the possibility of a Laurasian origin for Dragon Lizards (Fig. 3.2C; Grismer et al. 2016).

Here we make an effort to build upon the work of previous studies with a collaborative assembly of a novel dataset, containing 48 of 53 known genera, and 307 of ~440 known species of Dragon Lizards (Agamidae) and Butterfly Lizards (Leiolepidae). We use four genes regions (two mitochondrial fragments and two nuclear loci) and 13 acrodont fossils, to estimate the first time-calibrated agamid Tree of Life. Using our new dataset and the Gondwanan vicariant origin predictions of previous studies (Moody 1980; Macey et al. 1997, 2000; Schulte et al. 2003) as an initial null hypothesis, we sought to consider the possibility of an alternative biogeographic inference, namely Laurasian origin and dispersal into Gondwana for acrodonts, primarily Dragon Lizards of the family Agamidae. To explore the general alignment of natural history and biogeography in this clade, we used a fossil-calibrated phylogeny and ancestral state reconstructions analyses to reconstruct ancestral environments for acrodont ancestral lineages and the seven morphological groups statistically defined by Moody (1980). With the most comprehensive study on Dragon Lizard phylogeny to date, we use these initial analyses to reconsider long-held biogeographic expectations, and relate our findings to the diverse natural histories of agamid subfamilies.

Materials and Methods

Taxonomic Coverage and Fossil Calibrations

We compiled a Sanger dataset from previously published studies, for 307 species, representing all but five Dragon Lizard genera (*Psammophilus*, *Oriocalotes*, *Hypsicalotes*, *Cryptagama*, and *Psuedocophotis*), for two mitochondrial gene partitions (ND2 and 16S) and two nuclear loci (BDNF and RAG-1). We included the iguanid lizard genera *Oplurus, Anolis,* and *Basiliscus* as outgroups. We edited (as needed), aligned sequences, and constructed nexus files within Geneious Pro 5.0.4 (http://www.geneious.com, [Kearse et al. 2012]) for each individual locus. After reviewing the literature 23 described acrodont fossils, we narrowed our fossil calibration set down to the 13 most reliable identifiable calibration points (Table. 3.1).

Phylogenetic and Biogeographic Analyses:

Model fitting was performed on each locus using jModelTest 2.0 (Guindon and Gascuel 2003; Posada 2008); this procedure selected six-rate models of sequence evolution for the mitochondrial partitions and two-rate models for the nuclear loci (Table. 3.2). The selected models were implemented in preliminary Maximum Likelihood analyses using RAxML (Stamatakis 2014), with separate exploratory analyses performed on each locus to establish a lack of topological conflict. Once the once the lack of conflict among loci was established, we estimated phylogenetic relationships and divergence timescales for all families and subclades using Bayesian analyses in BEAST 2.3

(Bouckaert et al. 2014) with a four-partition dataset (ND2, 16S, BDNF, and RAG-1). Due to high amounts of missing data in the mitochondrial 16S region, we ran separate analyses with a reduced dataset of ND2, BDNF, and RAG-1. All Bayesian analyses were implemented with linked clock and tree models with Birth-Death and Fossilized Birth-Death (FBD) tree priors (Stadler 2010; Heath et al. 2014). The FBD prior allows for the placement of the fossils used for calibration as a tip in the phylogeny (Stadler 2010; Heath et al. 2014). The GTR+Γmodel was applied to the mitochondrial loci and HKY+Γto the nuclear loci and a relaxed uncorrelated lognormal clock model with an exponential prior for the mean rate of each partition was employed for all loci. Default values were used for all other priors and the analyses were run for 350 million generations sampling every 20,000 generations with chain stationarity and ESS values evaluated in Tracer 1.6 (Rambaut et al. 2014). The first 20% of trees were discarded as burn-in and a maximum clade credibility tree with median node heights was summarized using TreeAnnotator 2.3 (Bouckaert et al. 2014).

We assigned acrodont lineages to the following seven distinct biogeographic zones based on their present day distribution: Africa/Madagascar, Southwestern Asia, Central Asia, Mainland Asia (which includes Indo-Himalaya and Indochina), Sundaland, Wallacea, and Australia (Fig. 3.1). We then performed Bayesian ancestral-area estimations across the phylogeny using the R package BioGeoBears (Matzke, 2013a). BioGeoBears calculates maximum-likelihood estimates of the ancestral states at internal nodes by modeling transitions between geographical ranges along phylogenetic branches as a function of time. Because different ancestral-area reconstructions are based on different assumptions and can produce conflicting results (Matzke, 2013a, 2013b), we compared the dispersal–extinction–cladogenesis model (DEC), a likelihood implementation of dispersal–vicariance analysis (DIVALIKE), and the range-evolution model of the Bayesian binary (BAYAREALIKE) models. We also included a comparison of all models with and without the founder-event speciation parameter (+J), which can be added to any of these previously described models (Matzke, 2013b; and references therein) as a parameter to model descendant lineages colonizing a new area not inhabited by ancestral, parental types. We used the Akaike information criterion (AIC) to compare model fit (Matzke, 2013a; 2013b).

Ancestral State Reconstructions:

Moody (1980) assigned agamid and leiolepid genera to seven morphological and ecological groups, based on statistical analyses of 122 morphological characters; (1) terrestrial, (2) advanced terrestrial, (3) riparian semi-aquatic (including large bodied taxa), (4) general arboreal, (5) arboreal specialist (e.g. twig and canopy eco-morphologies), (6) ant-eater, and (7) generalist climber. In order to ascertain if there were secondary lines of evidence from the natural histories of Dragon Lizards that would distinguish between hypotheses, we coded all acrodont genera for their respective morphological group defined by Moody (1980) and reconstructed the ancestral morphological condition along with environmental preferences (tropical, arid, and temperate). R-scripts (R v3.2.0; R Core Team 2015) and the R libraries Ape (Paradis et al. 2004), Phytools (Revell 2012), and Geiger (Harmon et al. 2008) were used to first fit three continuous time Markov models of discrete trait evolution (single rate, equal rates, and all rates different) for the environmental preference and morphological group data. We then used Akaike information criterion to select the best-fit model of evolution for each dataset (Table. 3.3) and used selected models to perform ancestral reconstructions using Maximum Likelihood and Bayesian criteria on each of the trees from four- and three-gene datasets independently (Table. 3.3). We also executed additional reconstructions under the remaining models to explore the variation in the ancestral states and branch specific shifts between states. Maximum Likelihood estimations and Bayesian stochastic mapping (Nielsen 2002; Hulsenbeck 2003; Bollback 2006) were employed to infer the most likely state for each observed ancestor, and to observe shifts between states along individual branches, In our stochastic mapping analyses, we used default priors for the transition rate matrix (estimated form the data) and use the best-fit model of morphological evolution (discussed above) to simulated ancestral states over 10,000 trees.

Results

Our results compare with those of Macey et al. (2000), however within the Leiolepididae and the agamid subfamilies, our analyses recovered novel relationships not reported by previous studies (Figs. 3.3&3.4). The time-calibrated Bayesian analyses of the four gene and three gene multi-locus Sanger datasets returned phylogenies with the same higher-level relationships (Figs. 3.3&3.4), however minor differences within Agaminae and Draconinae were observed. Members of the Chamaeleonidae were recovered as sister to the Leiolepididae and the Agamidae (Figs. 3.3&3.4). The next lineage to diverge was a monophyletic Leiolepididae with a deep genetic divergence between the only two genera in the family, *Leiolepis* and *Uromystax* (Figs. 3.3&3.4). Agamidae was recovered as monophyletic, with Amphibolurinae sister to remaining radiations. Lastly, *Hydrosaurus* was placed as sister to the Agaminae and Draconinae

(Figs. 3.3&3.4) in all of the phylogeneies. As mentioned, we observed consistent differences in the relationships derived form these respective datasets, within the Agaminae and Draconinae. In all four-gene analyses, we recovered a polyphyletic *Laudakia* in the Agaminae and *Malayodracon* was placed as sister to the *Acanthosaura* group (node A: Figs. 3.3&3.5). In the three-gene analyses *Laudakia* was recovered as monophyletic and *Malayodracon* was placed as sister to the *Calotes* group and the *Acanthosaura* group (node A: Figs. 3.4&3.6). Additionally, the genus *Coryophlax* is only represented by the 16S locus, so its placement could only be inferred in the four-gene analyses.

Minor discrepancies in the placement of certain fossils were evident in our results of the four- versus three-gene calibration analyses (Figs. 3.3&3.4). The extinct subfamily Priscagamidae, represented by *Priscagama, Memiosaurus, Phrynosomimus* were placed as sister to *Uromystax* in the four-gene analyses and *Leiolepis* in the three-gene dataset (Figs. 3.3&3.4). Lastly, in the four-gene dataset *Protodraco* was placed as a stem lineage for all the members of the Draconinae with the exclusion of the extant genus *Mantheyus* (Fig. 3.3). However, in the analysis of the three-gene dataset *Protodraco* was recovered as the stem lineage to all members of the Draconinae (Fig. 3.4).

All BioGeoBears and BEAST 2.3 analyses resulted in similar estimations of ancestral areas and divergence times, with only minor conflicts in each of the subclades within the Leiolepididae and Agamidae (Figs. 3.3&3.4). For all ancestral-area analyses BAYAREALIKE + J was selected as the best-fit model (Table. 3.4), however the reconstructions under this model and the BAYAREALIKE model were not biologically justifiable (e.g. single areas estimated for all nodes in certain clades). Very similar likelihood scores were recovered for the next best class of models (DEC) and we used DEC+J in our ancestral area reconstructions.

Below, we will discuss timing of divergence for both our four- (Fig. 3.3), and three-gene datasets (Fig. 3.4). The BioGeoBears and BEAST 2.3 analyses indicate that acrodont lizards originated in Gondwana 168.0/166.6 MYA (four-gene divergence times/three-gene divergence times) on what is now Africa and Madagascar (Fig. 3.3), with a slight additional probability in mainland Asia (Fig. 3.4). The ancestor for the Agamidae and the Leiolepididae most likely originated in either Gondwana or Laurasia 138.8/156.6 MYA, being that they are distributed across what is now southwestern Asia, central Asia, and mainland Asia (Figs. 3.3&3.4). In this same area, the most recent common ancestor (MCRA) for the Leiolepididae may have also originated 128.0/150.6 MYA (Figs. 3.3&3.4). The ancestor of the Agamidae however, most likely originated exclusively in Laurasia across what we have defined as mainland Asia 130.7/148.4 MYA (Figs. 3.3&3.4). The ancestor for the Amphibolurinae is also estimated to be in mainland Asia and the shift to a Australian and Wallacean (Gondwanan) ancestor (node 1: Figs. 3.3&3.4) represents a dispersal from Laurasia to Gondwana. *Hydrosaurus* diverged from the Agaminae and the Draconinae in mainland Asia 130.0/146.0 MYA and is estimated to have originated 10.1/17.0 MYA in Wallacea, representing a second Laurasian dispersal into Gondwana (node 2: Figs. 3.3&3.4). The ancestor of the Agaminae and Draconinae diverged in mainland Asia 127.1/126.6 MYA and the Agaminae may have originated in Gondwana or Laurasia in what is now central Asia and mainland Asia 91.4/88.3 MYA (Figs. 3.3&3.4). Lastly, the Draconinae appears to have had origins in mainland Asia

118.1/92.4 MYA, and within the genus *Draco,* there is a third possible dispersal (node 3: Figs. 3.3&3.4) from Laurasia (Sundaland) into Gondwana (Wallacea).

For the Maximum Likelihood and Bayesian stochastic mapping of Moody's (1980) ancestral morphological/ecological, the most complex, all-rates different model was chosen as the most appropriate fit to the data. However, both the likelihood and AIC scores are only marginally superior (Table 3.3) therefore, we used the less parameter rich, two-rates model in all of our ancestral state analyses. Both analyses resulted in the same estimates of ancestral states for all ancestors in our phylogenies with minor conflict in the subclades (Figs. 3.3–3.6). Both criteria estimated that the ancestral condition for all acrodonts was a tropical, arboreal specialist (Figs. 3.3–3.6). We observed three independent shifts from tropical to arid environments within the Leiolepididae, Agaminae, and Amphibolurinae (Figs. 3.3–3.6). Within the Agamidae two shifts form this ancestral arboreal specialist to a riparian specialist (which could be considered a subcategory of an arboreal specialist being that the species of these genera live on trees exclusively at the water's edge) are inferred along the branches leading to *Physignathus* and the rest of the Amphibolurinae, and along the branch leading to *Hydrosaurus* (Figs. 3.3–3.6). The MCRA of the Amphibolurinae is estimated to be tropical and riparian but a shift to arid and temperate environments and an "advanced" terrestrial morphology is inferred at the ancestor of *Ctenophorus, Chlamydosaurus, Lophognathus, Amphibolurus, Gowidon, Rankinia, Diporiphora, Pogona,* and *Tympanocrptis* (Figs. 3.3–3.6). The MCRA for the Agaminae was most likely arid adapted and had a generalist climber morphology, whereas the ancestor of its sister lineage the Draconinae, was most likely tropical and an arboreal specialist (Figs. 3.3–3.6).

Discussion

Gondwanan and Laurasian Acrodont Biogeography

Our results allow us to accept a Gondwanan vicariant origin hypothesis for acrodont lizards consentient with Moody (1980) and Macey et al. (2000), and also allow us to reject a Gondwanan vicariant origin for the Dragon Lizards (Macey et al. 2000). Rejecting this hypothesis suggests that Dragon Lizards originated in Laurasia and subsequently dispersed across the Tethys Sea into Gondwana multiple times over the last 100 million years. During this time, the Tethys Sea was a mosaic of micro-continental blocks, smaller island archipelagos, and ophiolites that rifted from Gondwana during the Cretaceous and late Jurassic, towards Laurasia (Metcalf 2013 and reference therein). For some taxa, these landmasses may have served as refugia as they migrated across the Tethys Sea, and before colliding with Laurasia (DeBoer and Duffels 1996; Macey et al. 1997; Keogh 1998; Metcalf et al. 2001; Barker *et al.* 2002; Schulte et al. 2003). However, our data form multiple lines of evidence suggest that these landmasses may also have acted as stepping-stones, allowing for dispersal form Laurasia into Gondwana. First, within the Amphibolurinae, the Indochinese Water Dragon *Physignathus,* is sister to all Wallacean and Australian genera with an inferred ancestor in mainland Asia. Our results indicate that the ancestor of its sister taxon (all the Australian and Wallacean lineages) may have dispersed into Wallacea and Australia (node 1: Figs. 3.3&3.4). This is in opposition to previous interpretations, which suggested that *Physignathus* may have had a Gondwanan, vicariant origin and that its modern day distribution in Southeast Asia could have resulted from the collision of micro-continental blocks with Laurasia (Macey et al. 1997; Macey et al. 2000; Schulte et al. 2003).

Alternatively, we propose that Australian and Wallacean genera may be derived from "stepping stones" dispersal event into Gondwana across archipelagos of micro-continental blocks as they moved across Tethys Sea from Gondwana towards Laurasia during the mid Cretaceous. Second, the ancestor Asian Sail Fin Water Lizard genus *Hydrosaurus,* is estimated to have originated in Wallacea 10.1/17.0 MYA and diverged from a mainland Asian (Laurasian) ancestor 130/146.6 MYA (node 2: Figs. 3.3&3.4). These relationships and divergence times are consentient with a scenario of a Laurasian origins and subsequent dispersal into Gondwana across preexisting landmasses that dislodged from Gondwana in the late Jurassic 208 MYA (Metcalf 2011; Metcalf 2013). Lastly, within the Draconinae, the genus *Draco* has a monophyletic radiation on the island of Sulawesi in Wallacea (Gondwanan origin) whose ancestor diverged from a Sundaic or Wallacean lineage 52.8/47.0 MYA (Figs. 3.3&3.4); suggesting a possible third dispersal into Gondwana, across Wallace's line. Support for two of these three independent dispersals across the Tethys Sea (and Wallace's line) is provided by our ancestral state reconstructions of Moody's (1980) morphological/ecological groups. Our analyses provide evidence that two of these dispersal events we made by ancestors that were large-bodied, riparian, semi-aquatic species (nodes 1 & 2: Figs. 3.3–3.6). If these ancestors were morphologically and ecologically similar to their modern day descendants (riparian *Physignathus* and mangrove-specialist *Hydrosaurus*; both with a semiaquatic lifestyles), they may possibly have been pre-adapted for overwater dispersal (or rafting on mats of vegetation) across the Tethys Sea. The third dispersal event within *Draco* is a less straightforward. It is possible that the basal split within *Draco* is do a to a vicariant event across Wallace's Line, however it is conceivable that arboreal, canopy dwelling ecomorphologies may have been prone to distributing body weight while rafting on mats of vegetation. Thus, our inferred ancestral areas, natural histories, and divergence times are all are consentient with novel hypotheses of overwater “stepping stone” dispersal into Gondwana via micro-continental block archipelagos from the Cretaceous into the Miocene. This scenario should be more intensively evaluated with renewed attention to the direction of dispersal and explicit predictions derived from model based analyses, timing of diversification, and careful attention to the natural histories of the organism in question.

With the most taxonomically complete phylogeny of agamid lizards to date, a few novel relationships and biogeographic scenarios are worthy of discussion. The Amphibolurinae have been, and continued to be, very thoroughly studied (Cogger 2014 and references there in) and our analyses did not recover any new relationships within this clade. However, within the less well studied Draconinae and Agaminae, our analyses provided resolution of the systematic position of key taxa, and reveal new relationships bearing on long-contentious evolutionary and biogeographical hypotheses. Ours is the second study to recover a reciprocal monophyly between *Laudakia* and *Phrynocephalus* (Figs. 4&6) (Schulte and Moriano 2010). Although this relationship was only recovered in our analyses using the three-gene dataset (Figs. 4&6), we assume this to be an artifact relating to missing data mainly stemming from the 16S locus. Despite recovering all the Agaminae genera as monophyletic (three-gene dataset only), we did not recover strong support for many intrageneric relationships. This may relate to insufficient data, but could also conceivably be related to rapid radiation following a shift from tropical to arid environments (Figs. 5&6) and a quick filling of specialized climbing and terrestrial niches. It is worth noting that the genus *Phrynocephalus,* which are arid-adapted and exhibit the ant-eating morphology, are also excellent at partitioning terrestrial micro-habitats (e.g. sand vs. gravel: [Anderson 1999; Szczerbak 2003; Barabanov and Anajeva 2007; Sindaco & Jeremcenko 2008]), with unique radiations resulting from the formation each major plateau in Asia (Iranian, Pamir, and Tibetan) (Figs. 3.3&3.4).

The relationships and ancestral area reconstructions in the Draconinae are consentient with previous studies (Grismer et al. 2016), yet the addition of new Indian and Southeast Asian taxa brings new insight regarding previously tested hypotheses concerning Indian origins for agamid lizards. *Calotes minor* being nested within mainland Asian species of *Calotes* and the placement of *Coryophylax* as sister to the Sundaic endemic *Aphaniotis*, both provide additional evidence for faunal exchanges well after the hard collision of India and not just along the land bridges reported by Grismer et al. (2016). Additionally, the inclusion of the more Indochinese species of *Japalura* indicated that this genus is polyphyletic with two independent Indochinese radiations, one sister to the *Acanthosaura* group and the other sister to *Psuedocalotes*, and a single species sister, *J. planidorsata,* related to the Indian endemic *Salea* (Figs. 3&5). This suggests that the morphological characters having been used to group new agamid species into certain genera in Southeast Asia are misleading in some cases, suggesting that phylogeny-based taxonomic arrangements and use of multiple data types will have the highest probability of resulting in stable taxonomies. The placement of the Southeast Asian endemic *Malayodracon robinsonii* as monotypic genus and a historically contentious species of *Gonocephalus* (Denzer et al. 2015) exemplifies this point, and our analysis is the first study to place it phylogenetically and provide strong support from molecular data for its recognition as a distinct genus. The analyses of our four- and three-gene datasets produced conflicting placements of *Malayodracon* (node A: Fig. 3.3–3.6), both with low support. Our placement of *Malayodracon* indicate that it is an old lineage and may have been one of the first lineages to originate during the rapid radiation following India's initial contact with Sundaland (Grismer et al. 2016) and Grismer et al. (Chapter Two). However, to confirm this hypothesis and the accurate placement of *Malayodracon* should be approached with sequence capture data, such as those derived form ultra-conserved elements (Grismer et al. 2016).

Contentious Acrodont Fossil Calibrations: *A Review*

If used correctly, insights from the fossil record should broadly inform our knowledge of distribution patterns and improve the accuracy of modern day biogeographic hypothesis testing (Parham et al. 2012). Acrodont lizards have a rich fossil record composed of no fewer than 23 species (Gilmore 1943; Moody 1980; Averianov and Danilov 1996; Gao and Norell 2000; Wing et al. 2000; Smith et al. 2011; Gradstein et al. 2012; Head et al. 2013; Smith and Gauthier 2013). Although most are represented only by jaw fragments making modern-day genus and species comparisons difficult to impossible (Smith et al. 2009). Furthermore, given that having acrodont dentition is the main character used to identify and classify these fossils, confusion can result from the fact that not all lizards with this type of dentition are members of the clade *Acrodonta* (Moody 1980; Smith et al. 2011). For example, the fossils *Euposaurus* and *Homoeosaurus* were long considered stem acrodonts but after a comparison to a large series of squamate and other reptile fossils, it was determined they were rynchocephalians (Moody 1980). Additionally, the first acrodont fossil from the New

World was described from Brazil, potentially representing a key fossil calibration point for future analyses of acrodont evolution (Simoes et al. 2015). However, this fossil is only represented by jaw fragments and was diagnosed and placed phylogenetically on 12 dental characters. Determining whether this new fossil really is an acrodont, a rynchocephalian, or a new group of ancestral lizards with acrodont dentition will be a challenge for future studies. In this study, we avoided these pitfalls by elimination of any ambiguously identified fossils; however a full review of contentious acrodont fossils and reliable calibration points (Table. 3.1) for the acrodont families Leiolepididae and the Agamidae would be advisable.

The Leiolepididae has several reliable calibration points along the stem branch leading to the ancestor of *Leiolepis* and *Uromystax* as well as fossils that are excellent calibration points for the ancestors of each genus respectively (Figs. 3.3&3.4; Table. 3.1). However, the taxa *Phrynosomimus, Priscagama*, and *Mimeosaurus* all form the extinct subfamily the Priscagaminae (Figs. 3.3&3.4) and have been hypothesized to be the stem lineage to either *Leiolepis* or *Uromystax* (Gradstein et al. 2012). In our time calibrated analyses of the four and three-gene datasets, this subfamily was allowed to group along either of these branches depending on the dataset (Figs. 3.3&3.4). Given that fossil placement as a tip along a calibrated branch estimated from the sequence data (Stadler 2010; Heath et al. 2014) and by allowing this flexibility in the calibration point, the Priscagaminae was recovered as *Uromystax* in the four-gene dataset (Fig. 3.3) and stem to *Leiolepis* in the three-gene dataset (Fig. 3.4). I hypothesize the combination of the deep divergences separating the long branches leading to *Leiolepis* and *Uromystax,* conservative values used for the FBD priors, and the searching vagaries of the MCMC during the analysis, as contributing factors behind the inconsistent placement of this subfamily. Furthermore, despite living in different environments *Leiolepis* (tropical) and *Uromystax* (arid) have a similar terrestrial lifestyle resulting similar skeletal morphology (Peters 1971; Moody 1980; Smith et al. 2010). Therefore, we believe the original placement of this fossil subfamily based on the morphological examination by Borsuk-Bialynicka and Moody (1984) as stem to the entire Leiolepidiae and not to either genus is the most accurate and conservative placement of these fossils, and will need to be enforced in future analyses using these fossils. Additionally, it is likely that these same problems were complications in the placement of the stem *Uromystax, Uromastycinae, Paleochameleo, Barbatus* (Fig. 3.3&3.4: fossils 6, 7, and 8). Although these fossils were placed as stem to all *Uromystax* a priori, they were recovered along deeply divergent branches leading to both sister clades within *Uromystax* (Fig. 3.3&3.4: fossils 6, 7, and 8). If future analyses are to uses these three stem *Uromystax* fossils then precautions will need to be taken when setting the FBD priors and making the taxon sets for calibration points to ensure they are stem to both of these sister clades.

The amber fossil *Protodraco* was recently described from deposits in northwestern Myanmar and was shown to have foot morphology unique to the Indochinese endemic *Mantheyus* (Daza et al. 2016). Daza et al. (2016) hypothesized that *Protodraco* may represent a stem fossil of *Mantheyus* or to the entire Draconinae. Given the deep genetic divergence between *Mantheyus* and the remaining lineages, and that *Protodraco* and *Mantheyus* both were described from Indochina, either placement is justifiable as a calibration point for this fossil, however given the fossil currently is represented by very little material we recommend a conservative placement of

Protodraco as stem to the Draconinae.

Lastly, the fossil taxa *Vastanagama, Tinosaurus, Pleurodontagama, Bharatagama*, and *Geiseltaliellus* all have a combination of pleurodont dentition (similar to the Iguanidae) and Acrodont dentition. As a result, there has been confusion concerning the placement of these fossils and inconsistencies in their usage as calibration points (Smith et al. 2009; Reeder et al. 2015). These fossils all tend to have similar body plans and are distributed across Mongolia, North America, and Europe. Based on the fact that they are the only squamates that share this combination of dentition types; it is conceivable that they may be members of the same extinct radiation of lizards that had both pleurodont and acrodont dentition. The phylogenetic placement of these taxa with respect to extant linages should become more tractable once comparative osteological material from fossil and extant iguanids and acrodonts becomes available (Smith et al. 2009, Smith et al. 2010).

In this study we found support for a novel biogeographic interpretation, possibly overturning nearly half a century of agamid lizard biogeography, with its reliance on a Gondwanan vicariance as the only conceivable explanation (Brown, 2016) and the causal factor promoting diversification in this ancient clade of lizards. We provide new phylogeny-based evidence for multiple stepping-stone overwater dispersals from Laurasia back into Gondwana. These new hypotheses provide new insight into the complex and enigmatic biogeographic patterns inferred for this large diverse clade, and demonstrated how new data, the consideration of diverse data types and fossils, plus model based analyses, re-evaluation of previously considered intractable biogeographic hypotheses are possible. We also demonstrate how comparative analyses can enable researchers to address biogeographic hypotheses by incorporating natural history information via a statistical framework. We anticipate that future studies will involve/require the distinguishing among a plurality of methods, including using trait-based methods of biogeographic hypothesis testing (Vos et al. 2012).

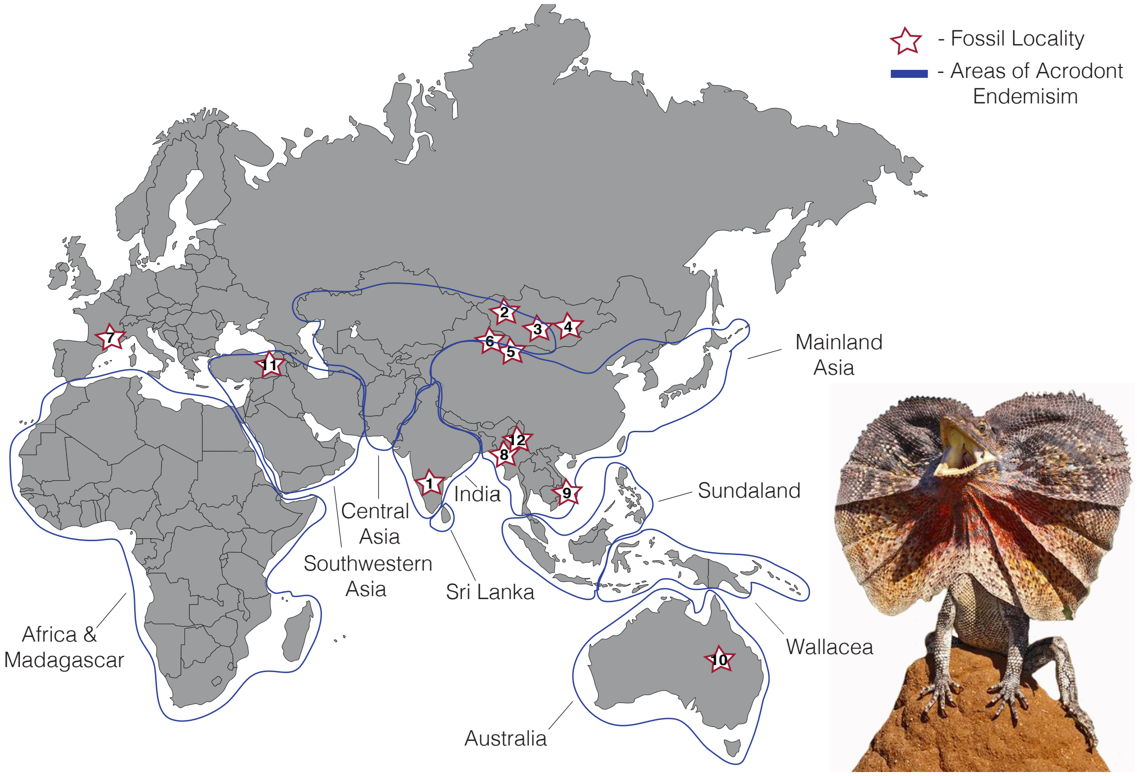

Figure 3.1– Map showing the distribution of Acrodont Lizard areas of endemism and the biogeographic areas use in our biogeographic analyses. The stars represent the locations of the fossils used as calibration point in this study. 1– *Acroiguana*; 2–*Phrynosomimus*; 3–*Priscagama*; 4–*Memiosaurus*; 5–*Leiolepidie*; 6–*Uromastxicae*; 7– *Barbatus*; 8–*Paleochameleo*; 9–*Physignathus*; 10–*Chlamydosaurus*; 11–*Phyrnocephalus*; 12–*Protodraco*

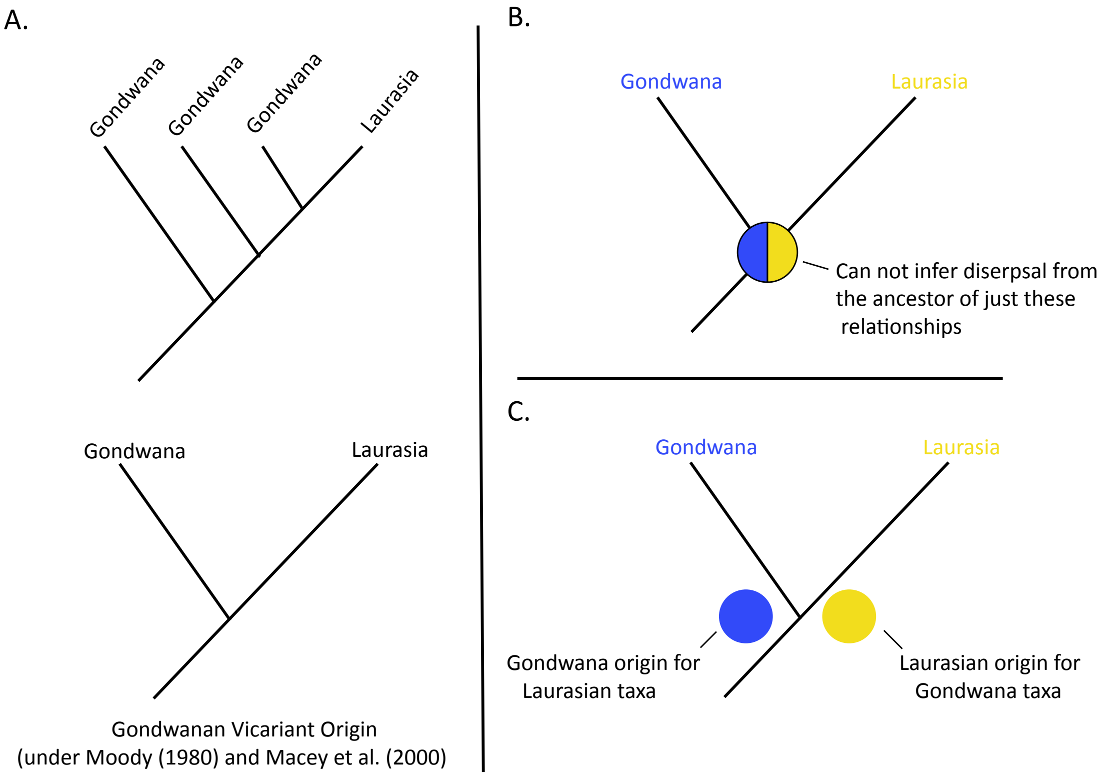

Figure 3.2– (A) The two expected topologies that would support a Gondwanan Origin for Laurasia species, proposed by Moody (1980) and Macey et al. (2000). (B) A hypothetical sister relationship between Gondwanan and Laurasia species, that we proposed that these relationships would not support a Gondwana origin dude to equal probability of each state at the ancestor. (C) A hypothetical sister relationship between Gondwanan and Laurasia species, where the ancestral distribution has been estimated within a broader taxonomic context and two inferred ancestral states needed to support a Laurasia or Gondwanan origin

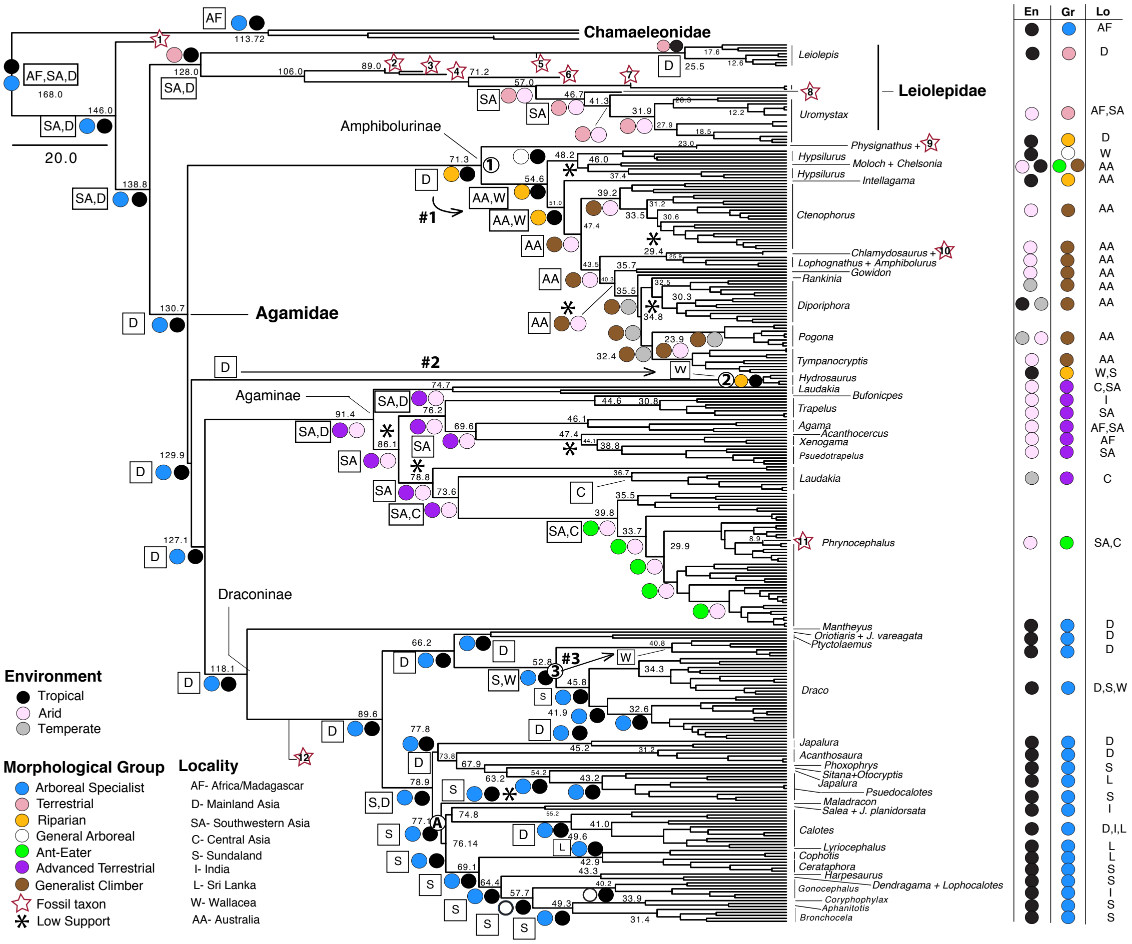

Figure 3.3– The tip based time-calibrated phylogeny of the four-gene dataset, with estimated divergence times for that node in MYA followed by the ancestral condition for environment, morphological group, and distribution. Colored circles represent the inferred ancestral condition for environment and morphological group, and the letters denote the ancestral distribution at each node. An asterisk indicates a relationship of low support. Fossils are: 1– *Acroiguana*, 2–*Phrynosomimus*, 3–*Priscagama*, 4–*Memiosaurus*, 5–*Leiolepidie*, 6–*Uromastxicae*, 7– *Barbatus*, 8–*Paleochameleo*, 9–*Physignathus*, 10–*Chlamydosaurus*, 11–*Phyrnocephalus*, 12–*Protodraco*.

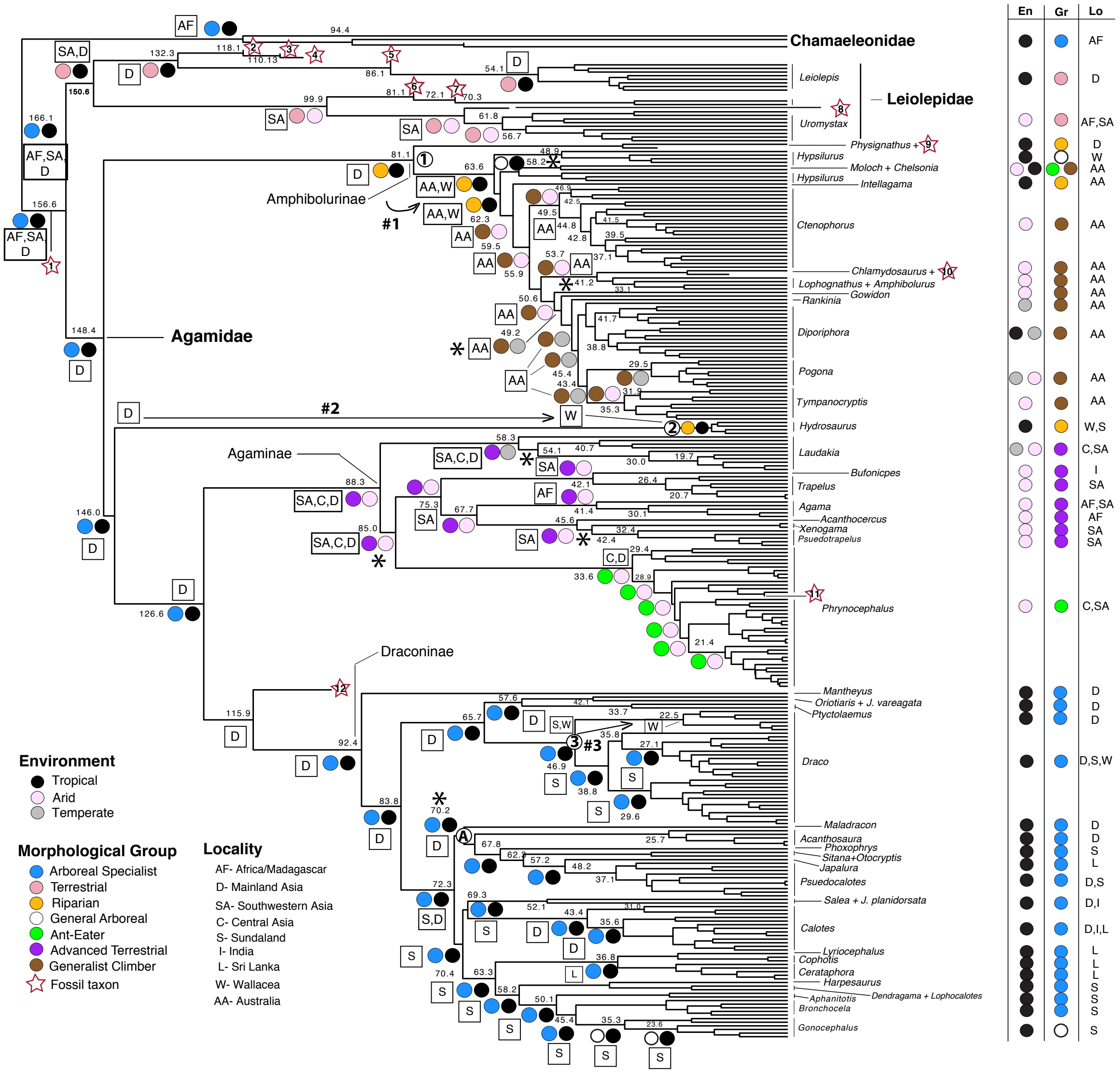

Figure 3.4– The tip based time-calibrated phylogeny of the three-gene dataset, with estimated divergence times for that node in MYA followed by the ancestral condition for environment, morphological group, and distribution. Colored circles represent the inferred ancestral condition for environment and morphological group, and the letters denote the ancestral distribution at each node. An asterisk indicates a relationship of low support. Fossils are: 1– *Acroiguana*, 2–*Phrynosomimus*, 3–*Priscagama*, 4–*Memiosaurus*, 5–*Leiolepidie*, 6–*Uromastxicae*, 7– *Barbatus*, 8–*Paleochameleo*, 9–*Physignathus*, 10–*Chlamydosaurus*, 11–*Phyrnocephalus*, 12–*Protodraco*.

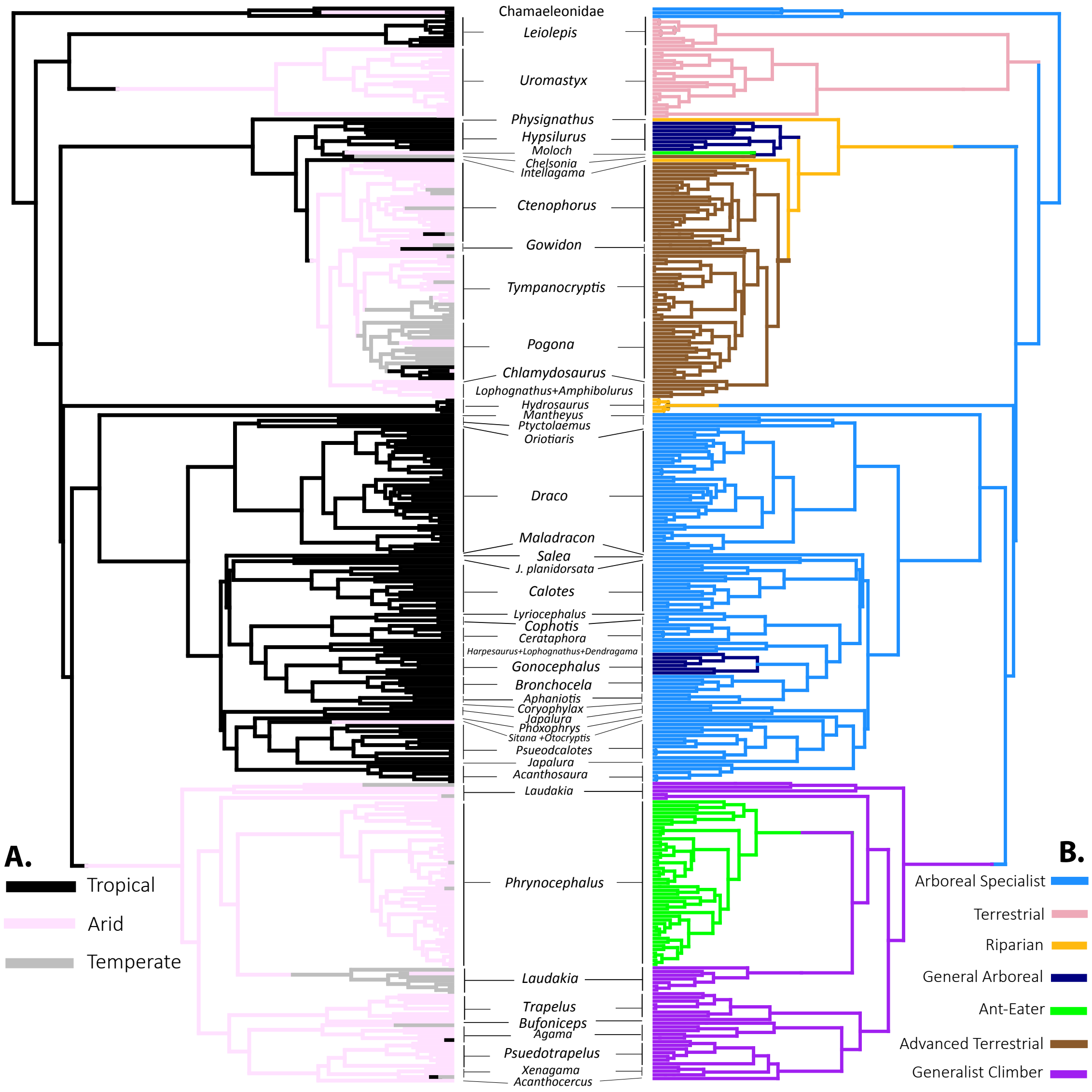

Figure 3.5– The results of the Bayesian stochastic mapping of the environmental data (right) and the morphological groups defined by Moody (1980) (left) on phylogeny of the tip based calibration analyses of the four-gene dataset.

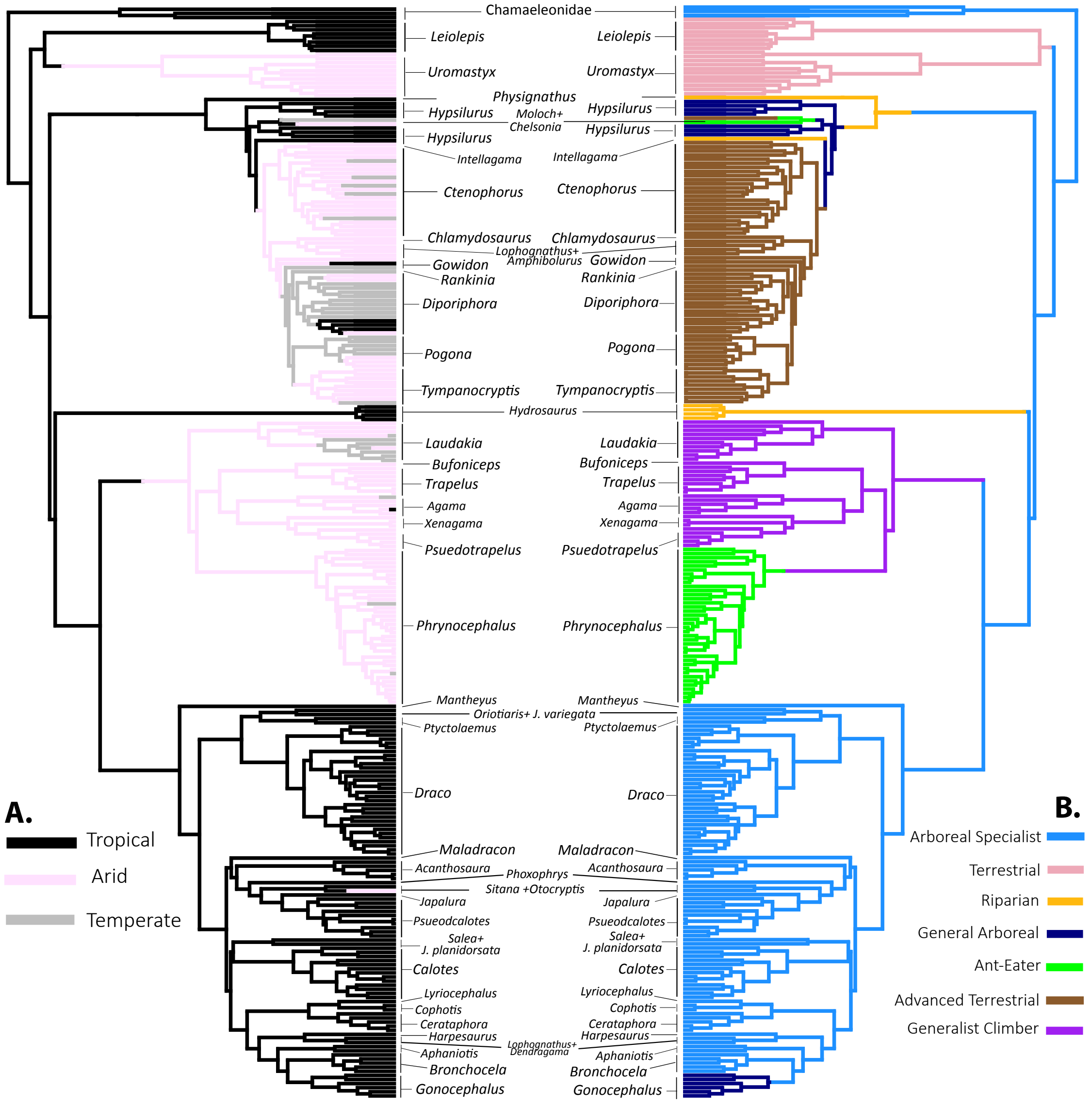

Figure 3.6– The results of the Bayesian stochastic mapping of the environmental data (right) and the morphological groups defined by Moody (1980) (left) on phylogeny of the tip based calibration analyses of the three-gene dataset.

Acrodont Fossil	**Age**	**Placement and Citation**
Fossil chameleonid with morphological similarities to *Rhampholeon*	18 MYA	Stem to *Rhampholeon* (Rieppel et al. 1992).
Mimeosaurus, Phrynosomimus, and *Pricagama* (Priscagamidae)	72–80 MYA	Sister to Lieolepidae (Gilmore 1943; Moody 1980; Gao, K., and M. A. Norell. 2000; Gradstein et al. 2012).
Leiolepidinae	53 MYA	Earliest stem for *Leiolepis* (Wing et al. 2000; Smith 2011; Smith and Gauthier 2013).
Uromastycinae	48 MYA	Earliest stem for *Uromastyx* (Averianov and Danilov 1996; Gradstein et al. 2012).
Paleochameleo	39 MYA	Reevaluated to be a stem lineage for *Uromastyx* (Moody 1980).
Barbatus	37 MYA	Stem for *Uromastyx* (Head et al. 2013)
Physignathus	19 MYA	Earliest possible stem for the genus (Covacevich et al. (1990)
Chlamydosaurus	18 MYA	Lydekker (1888)
Phrynocephalus	5 MYA	Earliest possible stem for the genus (Zerova and Chkhikvadze, 1984).
Protodraco	99 MYA	Daza et al. (2016)

Table 3.1 – A list of the Acrodont fossils used in this study along with their calibration points and ages.

Dataset	Gene	Model Selected	Model Used in BEAST
Four-Gene Dataset	ND2	GTR+Γ	GTR+Γ
	16S	GTR+Γ	GTR+Γ
	BDNF	K80	HKY+Γ
	RAG-1	HKY+Γ	HKY+Γ
Three-Gene Dataset	ND2	GTR+Γ	GTR+Γ
	BDNF	K80	HKY+Γ
	RAG-1	HKY+Γ	HKY+Γ

Table 3.2 – Loci and their associated estimated substitution models used in our phylogenetic analyses.

Dataset	Category	Model	Log likelihood
Four-Gene Dataset	Environment	One Rate	-128.18
		Two Rates	-112.59
		All Rates different	-108.21
	Morphological Group	One Rate	-61.22
		Two Rates	-51.92
		All Rates different	-45.14
Three-Gene Dataset	Environment	One Rate	-120.64
		Two Rates	-107.3
		All Rates different	-102.63
	Morphological Group	One Rate	-62.03
		Two Rates	-51.65
		All Rates different	-41.7

Table 3.3– Model fitting of three different continuous-time Markov models of discrete trait evolution used in our ancestral state estimations.

Dataset	Model	Log likelihood	Number of Parameters
Four-Gene Dataset	DIVALIKE	-411.18	2
	DIVALIKE+*j*	-399.46	3
	BAYAREALIKE	-386.63	2
	BAYAREALIKE+*j*	-343.42	3
	DEC	-387.04	2
	DEC+*J*	-376.62	3
Three-Gene Dataset	DIVALIKE	-352.34	2
	DIVALIKE+*j*	-346.67	3
	BAYAREALIKE	-350.01	2
	BAYAREALIKE+*j*	-313.08	3
	DEC	-338.24	2
	DEC+*J*	-329.95	3

Table 3.4– The results of BioGeoBEARS estimation of ancestral ranges, using a model selection approach to identify the appropriate biogeographical model for inference of range evolution across Acrodont Lizards.

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Appendices

Sample	Locality	ND2	Rag-1
Mantheyus phuwanensis	Laos	AY555836	FJ356735
Mantheyus phuwanensis	Laos	FMNH255495	
Mantheyus phuwanensis	Laos	FMNH262580	
Draco blanfordii	Vietnam	AF288242	JF806194
Draco blanfordii	Malaysia	LSUHC9427	
Draco maculatus	Malaysia	AF288248	
Draco maculatus	China	KUFS320	CAS210160
Draco spilopterus	Philippines	KU-ELR1338	
Ptyctolaemus collicristatus	Myanmar	AY555837	CAS219979
Ptyctolaemus collicristatus	Myanmar	CAS220561	
Ptyctolaemus gularis	Myanmar	AY555838	
Ptyctolaemus gularis	Myanmar	CAS221296	
Acanthosaura armata	Malaysia	LSUHC9351	
Acanthosaura lepidogaster	China	KU-CWL818	JF806187
Acanthosaura capra	Vietnam	AF128498	
Acanthosaura lepidogaster	Vietnam	AF128499	CAS206626
Phoxophrys nigrilabris	Malaysia	LSUHC4044	
Sitana ponticeriana	Sri Lanka	This study	
Otocryptis wiegmanni	Sri Lanka	AF128480	
Japalura flaviceps	China	AF128500	
Japalura splendida	China	AF128501	CAS214906
Pseudocalotes brevipes	Vietnam	AF128502	FMNH258703
Pseudocalotes larutensis	Malaysia	AF128503	
Pseudocalotes larutensis	Malaysia	LSUHC10285	
Pseudocalotes kingdonwardi	China	CAS242579	
Pseudocalotes microlepis	China	FMNH258710	FMNH258710
Salea horsfeldii	India	AF128490	AMB5739
Calotes chincollium	Myanmar	DQ289458	
Calotes emma	Thailand	DSM1256	
Calotes emma	Vietnam	AF128487	CAS215057
Calotes mystaceus	Thailand	DSM869	
Calotes mystaceus	Vietnam	AF128487	CAS207487
Calotes jerdoni	Myanmar	GQ502783	CAS219992
Calotes htunwini	Myanmar	DQ289463	
Calotes calotes	Myanmar	AF128482	AY662584
Calotes irawadi	Myanmar	DQ289468	
Calotes versicolor	Malaysia	LSUHC10327	
Calotes versicolor	Myanmar	AF128489	
Calotes ceylonensis	Sri Lanka	AF128483	
Calotes liocephalus	Sri Lanka	AF128484	
Calotes nigrilabris	Sri Lanka	AF128486	
Ceratophora aspera	Sri Lanka	AF128491	
Ceratophora stoddartii	Sri Lanka	AF128492	This study
Ceratophora tennentii	Sri Lanka	AF128521	
Lyriocephalus sculatus	Sri Lanka	AF128494	This study

Appendix 1 – List of all the species and their associated localities used in this study.

Cophotis ceylanica	Sri Lanka	AF128493	This study
Cophotis dumbara	Sri Lanka	GQ502785	
Aphaniotis fusca	Malaysia	AF128497	
Aphaniotis ornata	Indonesia	This study	
Bronchocela cristatella	Philippines	KU-BC1610	
Bronchocela cristatella	Philippines	ACD1547	
Bronchocela cristatella	Malaysia	AF128495	
Bronchocela marmoratus	Philippines	JAM981	
Bronchocela smaragdina	Cambodia	FMNH64815	HKV64815
Gonocephalus borneensis	Malaysia	ZFMK85275	
Gonocephalus grandis	Malaysia	AF128496	
Gonocephalus doriae	Malaysia	FM230175	F230175
Gonocephalus kuhli	Indonesia	ZFMK85969	
Gonocephalus interuptus	Philippines	RMB9384	
Gonocephalus sophae	Philippines	RMB8061	

Appendix 1 continued – List of all the species and their associated localities used in this study.

Acrodont Fossil	**Age**	**Placement and Citation**
Fossil chameleonid with morphological similarities to *Rhampholeon*	18 MYA	Stem to *Rhampholeon* (Rieppel et al. 1992).
Mimeosaurus and *Pricagama* (Priscagamidae)	72–80 MYA	Stem to *Leiolepis and Uromastyx* (Gilmore 1943; Moody 1980; Gao, K., and M. A. Norell. 2000; Gradstein et al. 2012).
Leiolepidinae	53 MYA	Earliest stem for *Leiolepis* (Wing et al. 2000; Smith 2011; Smith and Gauthier 2013).
Uromastycinae	48 MYA	Earliest stem for *Uromastyx* (Averianov and Danilov 1996; Gradstein et al. 2012).
Paleochameleo	39 MYA	Reevaluated to be a stem lineage for *Uromastyx* (Moody 1980).
Barbatus	37 MYA	Stem for *Uromastyx* (Head et al. 2013)
Physignathus	19 MYA	Earliest possible stem for the genus (Covacevich et al. (1990)
Phrynocephalus	5 MYA	Earliest possible stem for the genus (Zerova and Chkhikvadze, 1984).

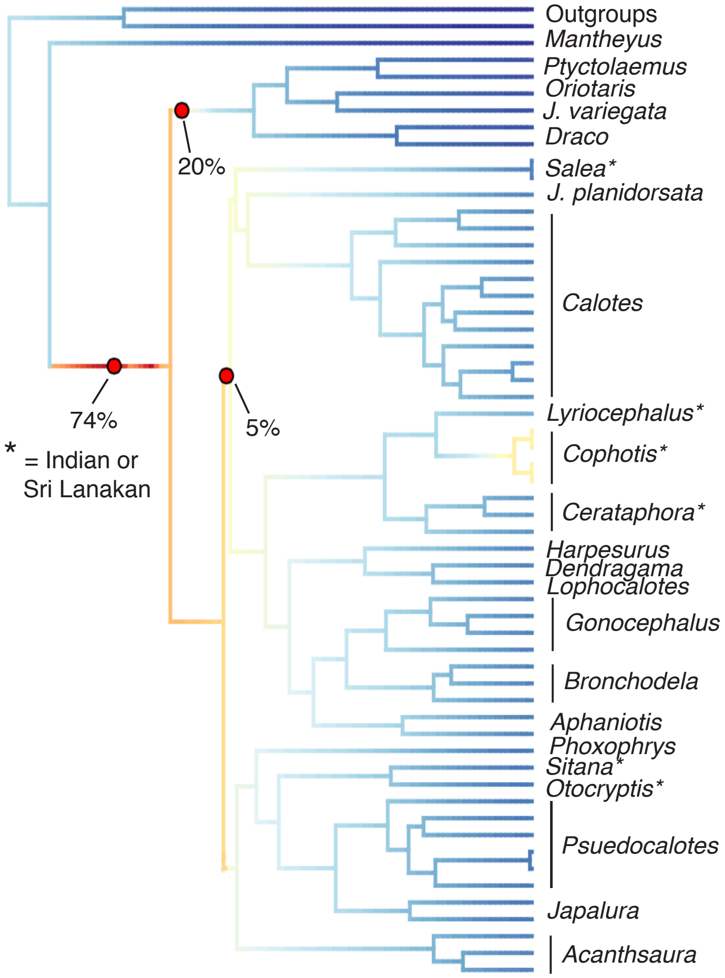
Appendix 2 – List of all the fossil calibrations, their ages, and their associated references, used in this study.

Sample	Locality	ND2	Rag-1
Mantheyus phuwanensis	Laos	AY555836	FJ356735
Draco blanfordii	Vietnam	AF288242	JF806194
Draco maculatus	China	KUFS320	CAS210160
Oriotari tricarinata	China	AF128478	This study
Ptyctolaemus collicristatus	Myanmar	AY555837	CAS219979
Ptyctolaemus gularis	Myanmar	CAS221296	
Acanthosaura armata	Malaysia	LSUHC9351	This study
Acanthosaura lepidogaster	China	KU-CWL818	JF806187
Acanthosaura bingtanensis	Malayasia	This study	This study
Phoxophrys nigrilabris	Malaysia	Grismer et al 2016	
Sitana ponticeriana	Sri Lanka	This study	
Otocryptis wiegmanni	Sri Lanka	AF128480	
Japalura flaviceps	China	AF128500	
Japalura splendida	China	AF128501	CAS214906
Japalura variegata	Nepal	AF128479	
Pseudocalotes brevipes	Vietnam	AF128502	FMNH258703
Pseudocalotes larutensis	Malaysia	AF128503	
Pseudocalotes floweri	Cambodia	Grismer et al 2016	
Pseudocalotes kakhiensis	China	Grismer et al 2016	
Pseudocalotes kingdonwardi	China	CAS242579	
Pseudocalotes microlepis	China	FMNH258710	FMNH258710
Salea horsfeldii	India	AF128490	AMB5739
Calotes chincollium	Myanmar	DQ289458	
Calotes emma	Vietnam	AF128487	CAS215057
Calotes mystaceus	Vietnam	AF128487	CAS207487
Calotes jerdoni	Myanmar	GQ502783	CAS219992
Calotes htunwini	Myanmar	DQ289463	
Calotes calotes	Myanmar	AF128482	AY662584
Calotes irawadi	Myanmar	DQ289468	
Calotes versicolor	Myanmar	AF128489	This study
Calotes ceylonensis	Sri Lanka	AF128483	
Calotes liocephalus	Sri Lanka	AF128484	
Calotes nigrilabris	Sri Lanka	AF128486	
Ceratophora aspera	Sri Lanka	AF128491	
Ceratophora stoddartii	Sri Lanka	AF128492	Grismer et al 2016
Ceratophora tennentii	Sri Lanka	AF128521	
Lyriocephalus sculatus	Sri Lanka	AF128494	Grismer et al 2016
Cophotis ceylanica	Sri Lanka	AF128493	Grismer et al 2016
Cophotis dumbara	Sri Lanka	GQ502785	
Aphaniotis fusca	Malaysia	AF128497	
Aphaniotis ornata	Indonesia	Grismer et al 2016	
Bronchocela cristatella	Malaysia	AF128495	
Bronchocela marmoratus	Philippines	JAM981	
Bronchocela smaragdina	Cambodia	FMNH64815	HKV64815
Gonocephalus borneensis	Malaysia	ZFMK85275	

Appendix 3 Sample list for Chapter 2.

Gonocephalus grandis	Malaysia	AF128496	
Gonocephalus doriae	Malaysia	FM230175	F230175
Gonocephalus kuhli	Indonesia	ZFMK85969	
Harpesaurus beccarii	Sumarta	ZFMK 48896	
Dendragama boulengeri	Sumarta	ZFMK 50532	
Lophocalotes ludekingi	Sumarta	ZFMK 46706	

Appendix 3 continued Sample list for Chapter 2.



Appendix S2 BAMM results.